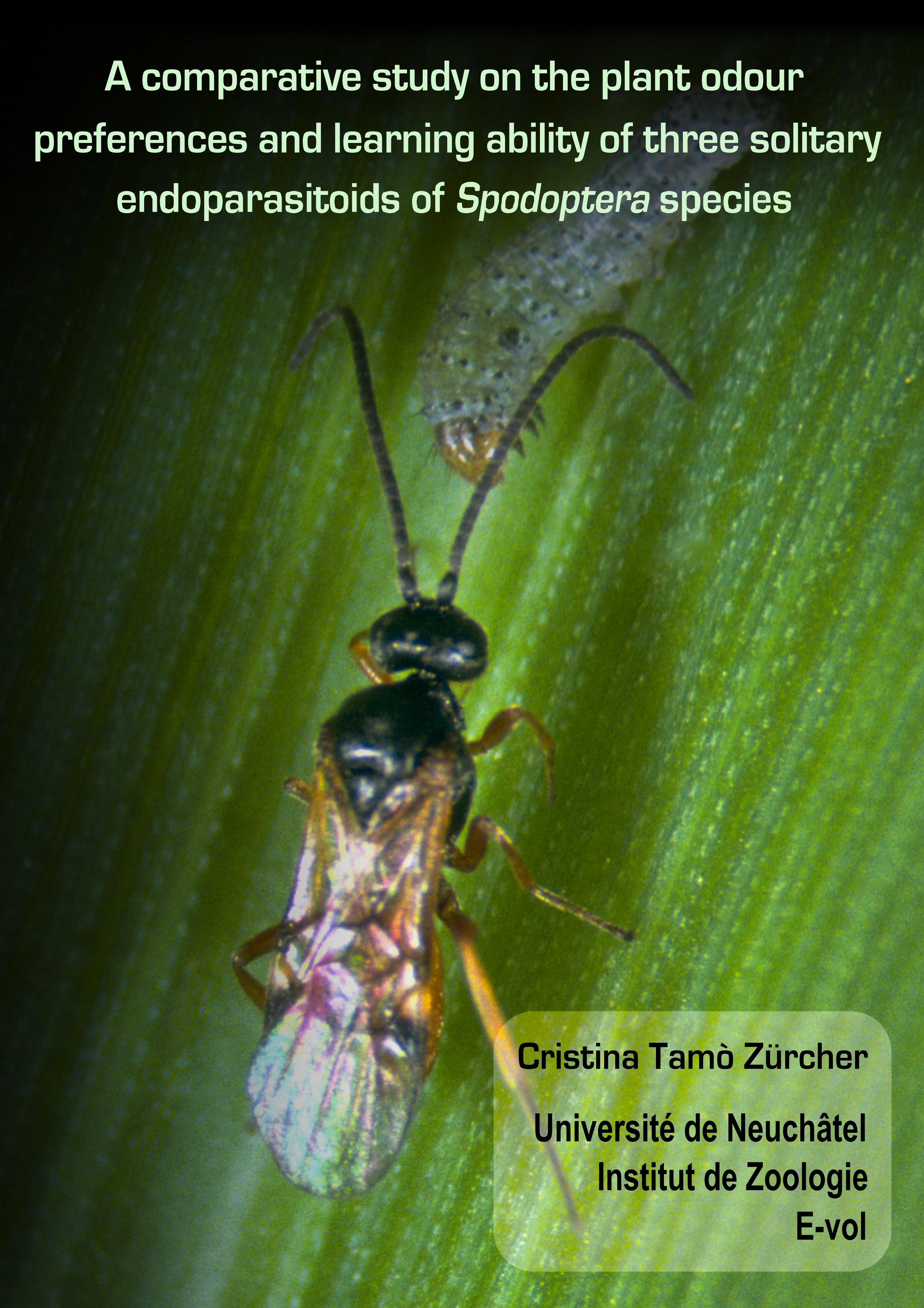


A comparative study on the plant odour preferences and learning ability of three solitary endoparasitoids of *Spodoptera* species

Cristina Tamò Zürcher

Université de Neuchâtel
Institut de Zoologie
E-vol

A comparative study on the plant odour preferences and learning ability of three solitary endoparasitoids of *Spodoptera* species

Thèse présentée à la Faculté des Sciences
Institut de Biologie
Université de Neuchâtel

Par

Cristina Tamò Zürcher

Acceptée sur proposition du jury:
Prof. Ted. C. J. Turlings, directeur de thèse
Prof. Dr. Louise E. M. Vet, rapporteur
Prof. Dr. Wolfgang Nentwig, rapporteur
Dr. Patrick Guerin, rapporteur

Soutenue le 23.01.2006

Université de Neuchâtel
2008

Université de Neuchâtel

FACULTE DES SCIENCES
Secrétariat-Décanat de la faculté
- Rue Emile-Argand 11
- CP 158
- CH-2009 Neuchâtel

IMPRIMATUR POUR LA THESE

A comparative study on the plant odour preferences and learning ability of three solitary endoparasitoids of *Spodoptera* species

Cristina Tamò-Zürcher

UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel,
sur le rapport des membres du jury

Mme L. Vet (NIOO, Heteren NL),
MM. T. Turlings (directeur de thèse), P. Guerin
et W. Nentwig

autorise l'impression de la présente thèse.

Neuchâtel, le 28 janvier 2008

Le doyen :
F. Kessler

Felix Kessler

Contents

Summary..**4**

Résumé...**7**

General Introduction...**10**

Chapter 1 A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping……………………………………………………………….**20**

Chapter 2 The role of chemical signals in mate finding by the parasitoids *Cotesia marginiventris* and *Microplitis rufiventrs*……………………………………….**32**

Chapter 3 A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours...**54**

Chapter 4 Effect of host diet and oviposition experience on host searching respectively and host acceptance behaviour of the parasitoid *Cotesia marginiventris*...**71**

Chapter 5 Relative importance of host derived and plant-derived volatiles in the attraction of the larval parasitoids *Cotesia marginiventris* and *Microplitis rufiventris*...**92**

Chapter 6 Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time saving foraging strategy..**117**

Conclusions and Outlook...**127**

Acknowledgments...**132**

Curriculum vitae..**134**

Summary

Keywords: herbivore-induced plant volatiles, olfactometer, parasitoid behaviour, learning, host finding, competition, host-discrimination

Odour cues are crucial for adult female and male parasitoids throughout their life span. The attraction of parasitoids to the odours of plants, hosts, food sources, conspecifics and competitors have been studied using various methods and tools, mostly in different types of olfactometers and wind tunnels. For this thesis we developed a novel olfactometer for high-through-put bio-assays. In chapter 1 this six-arm olfactometer is introduced and its usefulness tested. Six different odour sources can be offered in parallel and it permits simultaneous observation of insect attraction and odour trapping for subsequent chemical analyses. By testing the responses of the parasitoid *Cotesia marginiventris* to host-induced maize odours it is shown that this olfactometer is a very effective tool to study the attractiveness of single odours sources, but also the relative attractiveness of simultaneously offered odours. Moreover, the trapping of the induced plant volatiles during the behavioural assays was very effective and permitted to establish a direct correlation between the wasps' behaviour and the actual quantity and quality of the corresponding odours.

Not only host plants can be tested as odour sources, but also conspecific individuals of the same or of the opposite sex, permitting therefore to study the role of odours in another important aspect of the parasitoids behaviour, mate finding (Chapter 2). Males of the two parasitoid species *C. marginiventris* and *Microplitis rufiventris* were attracted to the odour of their conspecific females, whereas males were not attracted to other males, and females were not attracted to either sex. Unlike females, males of these two species were not found to be attracted to volatiles emitted by plants under attack by caterpillar hosts, also not after experiencing the odour during mating. Hence, males of both species are able to perceive and respond to female-produced pheromones, but they do not seem to make use of plant volatiles

for mate finding and do not use learning as a means to associate potential mates with an experienced odour. This is in contrast with the ability of females of many parasitoid species that are able to learn to associate specific plant-produced odours with the presence of hosts. In chapter 3 we compare the responses of naïve and experienced females of the three generalist larval endoparasitoid *C. marginiventris*, *M. rufiventris* and *Campoletis sonorensis* to the induced odours of three host plant species offered simultaneously. Despite their similarity in biology, these three parasitoids employ different foraging strategies and only *C. marginiventris* showed a clear use of associative learning as part of this strategy. In chapter 4 we tested if *C. marginiventris* also learns to associate odours with its host when it is immature. No such effect of so-called preimaginal learning was found for it, but in the same chapter it is shown that an ovipositional experience on one of four different substrates (three host plants and artificial diet) have an effect on the subsequent acceptance of hosts. Independently of the type of experience, *C. marginiventris* females started to parasitize faster and parasitized more when they were subsequently offered larvae feeding on one of the host plants, than when larvae were feeding on artificial diet. Only females that had an oviposition experience on artificial diet parasitized the larvae feeding on artificial diet as readily as on the plants. In Chapter 5 it is shown that, in order to find hosts, *C. marginiventris* and *M. rufiventris* make different use of volatiles resulting from fresh damage (green leaf volatiles), truly induced volatiles such as terpenoids and aromatics (which are only emitted several hours after initial damage by herbivores), and volatiles that are directly associated with the herbivores and/or their by-products. *C. marginiventris* is initially mostly attracted to odours from fresh damage and learns to use the induced volatiles after perceiving them during an encounter with hosts. *M. rufiventris* females, on the other hand, are initially attracted to plant volatiles, but when experiencing host on plants they appear to be less attracted to plant odours but respond to odours that are specifically emitted by the hosts or their products.

In the 6th and final chapter it is revealed that the odour of competing species can also affect the host searching behaviour of parasitoids. Studying the interactions between the two coexisting and competing species *C. marginiventris* and *C. sonorensis* it was found that the inferior intrinsic competitor *C. marginiventris* avoids the odour of the superior competitor *C. sonorensis*. This discrimination of habitats with and without competitors from a distance is expected to be an optimal foraging strategy.

Résumé

Mots-clés: volatiles induits par des herbivores, olfactomètre, comportement des parasitoïdes, apprentissage, recherche de l'hôte, compétition, discrimination de l'hôte

Les odeurs sont primordiales dans la vie des parasitoïdes aussi bien mâles que femelles. L'attraction des parasitoïdes pour les odeurs de plantes, d'hôtes, de nourriture, de conspécifiques et de compétiteurs a été étudiée grâce à de nombreuses méthodes, avec différents outils, en général au moyen d'olfactomètre ou de tunnels de vol. Dans ce travail, nous développons un nouvel olfactomètre qui permet un débit élevé. Dans le chapitre 1, cet olfactomètre à six bras vous sera présenté et son utilité démontrée. Six sources d'odeurs différentes peuvent être testées en parallèle et il permet simultanément l'observation de l'attraction des insectes et la collecte d'odeur pour les analyses. En testant les réponses du parasitoïde *Cotesia marginiventris* aux odeurs induites par l'hôte chez le maïs, nous montrons que cet olfactomètre est particulièrement efficace dans l'étude de l'attractivité d'une odeur particulière mais aussi de son attractivité relativement aux autres odeurs proposées. De plus, la capture pendant les expériences des volatils induits chez la plantes s'est avérée particulièrement efficace et a permis d'établir la corrélation entre le comportement des guêpes parasitoïdes et les caractéristiques quantitatives et qualitatives des odeurs testées.

Grâce à cet outil, il est aussi possible de tester les odeurs de conspécifiques du même sexe ou du sexe opposé, permettant ainsi d'étudier le rôle des odeurs dans un domaine particulièrement important de la vie de ces parasitoïdes, la recherche d'un partenaire sexuel (chapitre 2). Les mâles des deux espèces de parasitoïdes *C. marginiventris* et *Microplitis rufiventris* ont démontré leur attirance pour l'odeur des femelles de leur espèce ; les mâles n'ont pas montré d'attraction pour les individus de leur sexe et les femelles n'ont pas été attirée, ni par les mâles ni par les individus du même sexe. Contrairement aux femelles, les mâles de ces deux espèces n'ont pas été attiré par les volatils des plantes sous l'attaque des

chenilles même après avoir expérimenté un accouplement baigné de cette odeur. En outre, les mâles des deux espèces sont capable de percevoir et de répondre aux phéromones produites par les femelles mais ne semblent pas s'aider des volatils végétaux pour trouver leur partenaire, ni n'apprennent à associer la présence d'un partenaire potentiel avec une odeur donnée. Ce résultat est en contraste flagrant avec la capacité que démontrent les femelles de nombreux parasitoïdes d'associer des odeurs de plantes spécifiques avec la présence de leur hôte. Dans le chapitre 3, nous comparons les réponses des femelles des endoparasitoïdes *C. marginiventris*, *M. rufiventris* et *Campoletis sonorensis* naïves ou expérimentées à l'odeur induite chez trois types de plantes offertes simultanément. En dépit de leur similarités biologiques, ces trois espèces de parasitoïdes emploient des techniques de recherches bien différentes et c'est seulement *C. marginiventris* qui témoigne d'une claire utilisation de l' « associative learning » dans sa stratégie. Au chapitre 4, nous testons la faculté des stades immatures de *C. marginiventris* d'associer odeur et hôtes potentiels. Nous n'avons pas mis en évidence d'apprentissage pré-immaginal mais nous montrons que l'expérience d'oviposition sur l'un des quatre substrats (trois plantes hôtes et une diète artificielle) présente une importance sur l'acceptation future des différents hôtes. Indépendamment du type d'expérience, les femelles de *C. marginiventris* débutent leur parasitisme plus vite et parasitent plus lorsqu'on leur propose des larves se nourrissants sur l'une des trois plantes plutôt que sur le milieu artificiel. Seules les femelles ayant eu une expérience d'oviposition sur milieu artificiel ont parasité les larves se nourrissant sur milieu artificiel aussi vite que celles se nourrissants sur les plantes. Dans le chapitre 5, nous montrons que dans le but de trouver leur hôte, *C. marginiventris* et *M. rufiventris* font usage des volatils résultants des dommages frais (green leaf volatiles), des véritables volatils induits tels que terpenoïdes et composés aromatiques (qui sont émis plusieurs heures après l'attaque initiale des herbivores) et des volatils qui sont directement associés aux herbivores et à leur « by-products ». *C. marginiventris* est principalement attiré par l'odeur des dommages frais et apprends à utiliser les volatils induits après leur perception lors d'une rencontre avec un hôte. Au contraire, les femelles de *M. rufiventris* sont initialement attirées par les volatiles des plantes mais lorsqu'elles ont fait l'expérience d'une rencontre avec un hôte sur la plante, elles semblent être moins attirées par les volatils de plantes mais répondent plus aux odeurs qui sont émises spécifiquement par l'hôte et ses produits.

Dans le chapitre 6 et le chapitre final, nous révélons que l'odeur d'une espèce compétitrice peut aussi affecter la recherche de l'hôte chez ces parasitoïdes. En étudiant les interactions entre deux espèces compétitrices et coexistant en un même endroit, *C. marginiventris* et *C. sonorensis*, nous avons trouvé que le compétiteur intrinsèquement inférieur *C. marginiventris* évite les odeurs produites par le compétiteur supérieur *C sonorensis*. La discrimination à distance de l'habitat avec ou sans compétiteur est pressentie comme une stratégie de recherche optimale.

Traduction Nicolas Margraf

General Introduction

Females of various parasitoids of herbivores make use of plant odours induced by insect feeding to locate host plants that may carry their hosts (Vet and Dicke, 1992; Turlings and Benrey, 1998; Dicke and Vet, 1999; Turlings and Wäckers, 2004). Several of these parasitoid species have been shown to be able to learn the plant and other odours when perceiving them during an encounter with hosts (Turlings et al., 1993; Vet et al., 1995).

This ability of associative learning is generally expected to be an adaptive strategy for parasitoids that have a broad host range or which can find their hosts on multiple plant species (Vet and Groenewold, 1990; Vet and Dicke, 1992; Vet, 1999; Steidle and van Loon, 2003), but the general validity of this concept is unclear (Steidle and van Loon, 2003).

Similarly, preimaginal experiences with odours from food plants during the development inside the host can affect the wasps' behaviour as adults, demonstrating an effect of the host's food plant on the behaviour of an adult wasp (Monteith, 1958; Vet, 1983; Drost et al., 1986; Drost et al., 1988; Hérard et al., 1988; Wickremasinghe and van Emden, 1992; Cortesero and Monge, 1994; Bogahawatte and van Emden, 1996; Storeck et al., 2000; Gandolfi et al., 2003).

Other studies show that an oviposition experience in hosts on a particular substrate not only increases the attractiveness of the odours produced by this substrates, but also increases the acceptance of the hosts subsequently encountered on the same food substrate, which may directly affect their reproductive success (Vet and Schoonman, 1988; Mattiacci et al., 2000).

Induced plant odours are not the only attractive odours for adult parasitoids. Adult males parasitoids belonging to many species are known to make use of volatile sex pheromones produced by their conspecific females in order to optimize their reproductive success (for reviews see Eller et al., 1984 and Fauvergue et al., 1999). The possible exploitation of plant odours by male parasitoids in mate finding has only rarely been studied. In the few studies that looked at this, males mostly failed to respond to plant cues (Eller et al., 1988; Messing and Jang, 1992; Udayagiri and Jones, 1992; Jones, 1996; Pettersson et al., 2000; Pettersson, 2001). It remains unclear, however, if males can learn to use plant-provided cues to find mate, in the same way females do to find host.

Different parasitoids species are associated with one or more hosts and this co-dependence on the same resource leads to competition among them. Interspecific host discrimination (avoidance of multiparasitism) is rarely found, and van Alphen and Visser (1990) argue that it could eventually evolve only in inferior competitors. Based on a simulation model, (Turlings et al., 1985) suggest that, as long as a female parasitoid is not egg limited, she should always accept a host already parasitized by another species, even if the chance of offspring survival is small. This assumption would change if host rejection would result in considerable time gain, time that could be used to find more suitable hosts (Turlings et al., 1985). Such a time gain could be achieved if already parasitized hosts are detected and rejected from a distance. Interestingly, it has been shown that odours produced by parasitoid belonging to a certain species or induced by their presence can serve as signal for other co-occurring and competing parasitoid species (Janssen et al., 1995a; 1995b) and this could be see as a strategy to avoid interspecific competition.

To elucidate the specific questions concerning parasitoid attraction or repellence by various odour sources, many laboratory studies have been conducted using mostly different types of olfactometers and wind tunnels. The most commonly used olfactometer are the Y-shaped (Mc Indoo 1926, Snapp and Swingle, 1929), the T-shaped linear tract (Sakuma and Fukami, 1985) and the four arm olfactometers (Pettersson, 1970 and Vet et al., 1983). Chemical information on the odour sources had normally to be obtained in separate analytical studies. This has become a hurdle with the increasing need to compare large numbers of odour sources for their relative attractiveness and to understand which compounds are the most important for the attraction of the insects. The composition of the induced volatiles emitted by herbivore damaged plants vary considerably between different plant species and between different genotypes of the same plant species, revealing differences in the quantity as well as the quality (relative ratios) of the produced volatiles blends (for reviews see Dicke, 1994; Dicke, 1999). Among plants belonging to the same species, considerable variability can also be found under different abiotic conditions, for different herbivore species and herbivore stages (Gouinguene et al., 2001). Moreover, the induced odour emissions can, for the same species, differ over time, showing some compounds immediately after damage and others needing more time to be synthesised by the attacked plants. These differences of the blend composition were often found to be reflected in the different attractiveness of the plant species or varieties for parasitoids, which was not only due to differences in quantity, but also to qualitatively differences (Hoballah et al., 2002). To investigate the consequences of all this variation for parasitoid attraction, more efficient bio-assay methods need to be developed.

Thesis outline

For this thesis we first developed an novel bio-assay tool and with that I investigated several of the above questions concerning the use of volatile substances by parasitoids with a study system that consisted of the three plant species maize (*Zea mays*, var. Delprim), cotton (*Gossypium herbaceum*) and cowpea (*Vigna unguiculata*); the herbivores Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae); and the larval parasitoids *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), *Microplitis rufiventris* (Kokujev) (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Cameron) (Hymenoptera; Ichneumonidae). These three parasitoids are important enemies of important lepidopteran pests including several *Spodoptera* spp.

The thesis is organized as follows:

Chapter 1 introduces a six-arm olfactometer that was developed to facilitate our efforts to determine which specific compounds in the plant provided signals are the key attractants to parasitoids. For this purpose we wanted to test multiple odour sources at once. The principle aim of the chapter was to test if this six-arm olfactometer is an effective tool that permits us to test the relative attractiveness of different odour sources offered to the wasps at the same time. An additional feature of the device is the possibility to collect volatiles from the different odour sources during the bioassays. It was tested if indeed the composition and quantities of the collected volatile blends could be determined after each experiment by analysing the collected samples by gas chromatography and mass spectroscopy. We further studied if, in the olfactometer, wasps could distinguish between several odour sources if they were offered simultaneously and if wasps that were released together affect each other's choices.

In Chapter 2 we tested the possible role of sex pheromones and/or plant volatiles in mate finding by *C. marginiventris* and *M. rufiventris*. Considering that the wasps are solitary and likely to emerge from their cocoons away from potential mates, it can be expected that they use cues like sex pheromones to locate such mates. To see if this is the case, we observed responses of females and males to the odours of individuals of the same or the opposite sex. We further explored the possibility that plant volatiles are exploited by males in order to locate females and used he olfactometer to test this, whereby we also considered the possibility that males may learn to associate plant volatiles with the presence of females.

In Chapter 3 we used the six-arm olfactometer to compare the responses of the three larval endoparasitoid, *Cotesia marginiventris*, *Microplitis rufiventris* and *Campoletis sonorensis*, to the induced odours of three plant species: maize, cowpea, and cotton, that can all be attacked by their herbivorous hosts. We had clear evidence from the literature and our own observations that the three species use such cues to locate hosts, but also had indications that they differ in how they change their responses after they experience specific odours during a contact with hosts. To test this, the preference of naïve females for the plant induced odours were compared with the preference of females that had an oviposition experience with one of the plant-hosts-complex to find out if the adult females of these three species showed an effect of associative learning.

In Chapter 4 we tested if the responses to induced plant odours in females of *C. marginiventris* are affected by the diet that was eaten by the host in which they developed as a larva. So-called preimaginal conditioning through host diet has been claimed for one parasitoid and learning of odours during emergence from the cocoon has been observed in several. To find out if this might be the case also for *C. marginiventris* females they were reared in hosts on either artificial diet or maize, cotton or cowpea leaves and then tested and compared in a six-arm olfactometer in which the induced odours of the three plant species were offered simultaneously. In the same chapter we also tested if an oviposition experience in hosts on a particular substrate (maize leaves, cotton leaves, cow leaves or artificial diet) affects their host acceptance behaviour and parasitism rate during a subsequent contact with hosts on the same or a different substrate.

In Chapter 5 we tested the relative importance of host-derived and plant-derived volatiles for the attraction of the parasitoids *C. marginiventris* and *M. rufiventris*. This was done by comparing responses of both naïve and experienced *C. marginiventris* and *M. rufiventris* to freshly damaged plants and plants with older damage, as well as to volatiles emitted from host larvae and /or their frass.

In Chapter 6 we tested the hypothesis that competing wasps may have adapted their foraging behaviour to optimize their foraging success. The outcome of competition between the two sympatric parasitoids *C. marginiventris* and *C. sonorensis* was studied by analysing the intrinsic competition in multiparasitised larvae and we tested if, in accordance with the hypothesis, the inferior competitor could recognize and would avoid the odour of the superior competitor.

References

Bogahawatte, C. N. L. and van Emden, H. F. (1996). The influence of the host plant of diamond-back moth (*Plutella xylostella*) on the plant preferences of its parasitoid *Cotesia plutellae* in Sri Lanka. *Physiological Entomology* 21, 93-96.

Cortesero, A. M. and Monge, J. P. (1994). Influence of preemergence experience on response to host and host-plant odors in the larval parasitoid *Eupelmus vuilleti*. *Entomologia Experimentalis et Applicata* 72, 281-288.

Dicke, M. (1994). Local and systemic production of volatile herbivore-induced terpenoids - their role in plant carnivore mutualism. *Journal of Plant Physiology* 143, 465-472.

Dicke, M. (1999). Specificity of herbivore-induced plant defences. In *Insect-plant interactions and induced plant defence*, (eds. Chadwick D. J. and Goode J. A.), pp. 43-54. Novartis Foundation, Chichester: John Wiley and Sons.

Dicke, M. and Vet, L. E. M. (1999). Plant-carnivore interactions: evolutionary and ecological consequences for plant, herbivore and carnivore. In *Herbivores: between plants and predators.*, (eds. H. Olff V. K. Brown and R. H. Drent), pp. 483-520. Oxford: Blackwell Science.

Drost, Y. C., Lewis, W. J. and Tumlinson, J. H. (1988). Beneficial arthropod behavior mediated by airborne semiochemicals.5. Influence of rearing method, host plant, and adult experience on host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis*. *Journal of Chemical Ecology* 14, 1607-1616.

Drost, Y. C., Lewis, W. J., Zanen, P. O. and Keller, M. A. (1986). Beneficial arthropod behavior mediated by airborne semiochemicals.1. Flight behavior and influence of preflight handling of *Microplitis croceipes* (Cresson). *Journal of Chemical Ecology* 12, 1247-1262.

Eller, F. J., Bartelt, R. J., Jones, R. L. and Kulman, H. M. (1984). Ethyl (Z)-9-hexadecenoate a sex-pheromone of *Syndipnus rubiginosus*, Hymenoptera, Ichneumonidae a sawfly parasitoid. *Journal of Chemical Ecology* 10, 291-300

Eller, F. J., Tumlinson, J. H. and Lewis, W. J. (1988). Beneficial arthropod behavior mediated by airborne semiochemicals.2. Olfactometric studies of host location by the parasitoid

Microplitis croceipes (Cresson) (Hymenoptera, Braconidae). *Journal of Chemical Ecology* 14, 425-434.

Fauvergue, X., Fleury, F., Lemaitre, C. and Allemand, R. (1999). Parasitoid mating structures when hosts are patchily distributed: field and laboratory experiments with *Leptopilina boulardi* and *L. heterotoma*. *Oikos* 86, 344-356.

Gandolfi, M., Mattiacci, L. and Dorn, S. (2003). Preimaginal learning determines adult response to chemical stimuli in a parasitic wasp. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270, 2623-2629.

Gouinguene, S., Degen, T. and Turlings, T. C. J. (2001). Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology* 11, 9-16.

Hoballah, M. E. F., Tamò, C. and Turlings, T. C. J. (2002). Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: Is quality or quantity important? *Journal of Chemical Ecology* 28, 951-968.

Hérard, F., Keller, M. A., Lewis, W. J. and Tumlinson, J. H. (1988). Beneficial arthropod behavior mediated by airborne semiochemicals.4. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson (Hymenoptera, Braconidae). *Journal of Chemical Ecology* 14, 1597-1606.

Janssen, A., van Alphen, J. J. M., Sabelis, M. W. and Bakker, K. (1995a). Odor-mediated avoidance of competition in Drosophila parasitoids - the ghost of competition. *Oikos* 73, 356-366.

Janssen, A., van Alphen, J. J. M., Sabelis, M. W. and Bakker, K. (1995b). Specificity of odor mediated avoidance of competition in *Drosophila* parasitoids. *Behavioral Ecology and Sociobiology* 36, 229-235.

Jones, R. L. (1996). Semiochemicals in host and mate finding behavior of *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae). *Florida Entomologist* 79, 104-108.

Mattiacci, L., Hutter, E., Schoch, D., Scascighini, N. and Dorn, S. (2000). Plant-odour mediates parasitoid host handling and oviposition in an endophytic tritrophic system. *Chemoecology* 10, 185-192.

Mc Indoo, N.E. (1926). An insect olfactometer. *Journal of Economic Entomology* 19, 545-571.

Messing, R. H. and Jang, E. B. (1992). Response of the fruit-fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera, Braconidae) to host-fruit stimuli. *Environmental Entomology* 21, 1189-1195.

Monteith, L. G. (1958). Influence of food plant of host on attractiveness of the host to tachinid parasites with notes on preimaginal conditioning. *Canadian Entomologist* 90, 478-482.

Pettersson, J. (1970). An aphid sex attractant. I. Biological studies. *Entomologica Scandinavica* 1, 63-73.

Pettersson, E. M. (2001). Volatile attractants for three Pteromalid parasitoids attacking concealed spruce bark beetles. *Chemoecology* 11, 89-95.

Pettersson, E. M., Sullivan, B. T., Anderson, P., Berisford, C. W. and Birgersson, G. (2000). Odor perception in the bark beetle parasitoid *Roptrocerus xylophagorum* exposed to host associated volatiles. *Journal of Chemical Ecology* 26, 2507-2525.

Sakuma, M. and Fukami, H. (1985). The linear track olfactometer - an assay device for taxes of the german cockroach, *Blattella-Germanica* (L) (Dictyoptera, Blattellidae) toward their aggregation pheromone. *Applied Entomology and Zoology* 20, 387-402.

Snapp, O.I. and Swingle, H.S. (1929). Further results with the McIndoo olfactometer. *Journal of Economic Entomology* 22, 984-985.

Steidle, J. L. M. and van Loon, J. J. A. (2003). Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomologia Experimentalis et Applicata* 108, 133-148.

Storeck, A., Poppy, G. M., van Emden, H. F. and Powell, W. (2000). The role of plant chemical cues in determining host preference in the generalist aphid parasitoid *Aphidius colemani*. *Entomologia Experimentalis et Applicata* 97, 41-46.

Turlings, T. C. J. and Benrey, B. (1998). Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience* 5, 321-333.

Turlings, T. C. J., van Batenburg, F. D. H. and van Strien van Liempt, W. (1985). Why is there no interspecific host discrimination in the 2 coexisting larval parasitoids of Drosophila species - *Leptopilina heterotoma* (Thomson) and *Asobara tabida* (Nees). *Oecologia* 67, 352-359.

Turlings, T. C. J., Waeckers, F., Vet, L. E. M., Lewis, W. J. and Tumlinson, J. H. (1993). Learning of host-finding cues by hymenopterous parasitoids. In *Insect Learning: Ecological and Evolutionary Perspectives*, eds. D. R. Papaj and A. Lewis), pp. 51-78. New York: Chapman & Hall.

Turlings, T. C. J. and Wäckers, F. L. (2004). Recruitment of predators and parasitoids by herbivore-damaged plants. In *Advances in Insect Chemical Ecology*, eds. R. T. Cardé and J. Millar), pp. 21-75: Cambridge University Press.

Udayagiri, S. and Jones, R. L. (1992). Flight behavior of *Macrocentrus grandii* Goidanich (Hymenoptera, Braconidae), a specialist parasitoid of European Corn-Borer (Lepidoptera, Pyralidae) - factors influencing response to corn volatiles. *Environmental Entomology* 21, 1448-1456.

van Alphen, J. J. M. and Visser, M. E. (1990). Superparasitism as an adaptive strategy for insect parasitoids. *Annual Review of Entomology* 35, 59-79.

Vet, L. E. M. (1983). Host-habitat location through olfactory cues by *Leptopilina clavipes* (Hartig) (Hym, Eucoilidae), a parasitoid of fungivorous Drosophila - the influence of conditioning. *Netherlands Journal of Zoology* 33, 225-248.

Vet, L.E.M., Vanlenteren, J.C., Heymans, M., & Meelis, E. (1983). An air-flow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiological Entomology* 8, 97-106.

Vet, L. E. M. (1999). From chemical to population ecology: Infochemical use in an evolutionary context. *Journal of Chemical Ecology* 25, 31-49.

Vet, L. E. M. and Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* 37, 141-172.

Vet, L. E. M. and Groenewold, A. W. (1990). Semiochemicals and learning in parasitoids. *Journal of Chemical Ecology* 16, 3119-3135.

Vet, L. E. M., Lewis, W. J. and Cardé, R. T. (1995). Parasitoid foraging and learning. In *Chemical Ecology of Insects 2*, eds. R. T. Cardé and W. J. Bell), pp. 65-101. New York: Chapman & Hall.

Vet, L. E. M. and Schoonman, G. (1988). The influence of previous foraging experience on microhabitat acceptance in *Leptopilina heterotoma*. *Journal of Insect Behavior* 1, 387-392.

Wickremasinghe, M. G. V. and van Emden, H. F. (1992). Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. *Physiological Entomology* 17, 297-304.

Chapter 1

A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping

Based on: Ted C. J. Turlings, Ted C. J. Turlings and Cristina Tamò, A. A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours, *Physiological Entomology* (2004) 29, 45-55.

Physiological Entomology (2004) **29**, 45–55

A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping

TED C. J. TURLINGS, A. C. DAVISON† and CRISTINA TAMÒ
Laboratory of Animal Ecology and Entomology, University of Neuchâtel, Switzerland and †Institute of Mathematics, Swiss Federal Institute of Technology, Lausanne, Switzerland

Abstract. Behavioural assays to study insect attraction to specific odours are tedious, time consuming and often require large numbers of replications. Olfactometer and flight tunnel tests can usually only be conducted with one or two odour sources at a time. Moreover, chemical information on the odour sources has to be obtained in separate analytical studies. An olfactometer was developed in which six odours can be tested simultaneously for their relative attractiveness while during the assays, part of each test odour can be trapped for further analyses. The effectiveness of this six-arm olfactometer was tested by observing the responses of the solitary endoparasitoid *Cotesia marginiventris* (Cresson) to host-induced odours from young maize plants. For statistical analyses, we used log-linear models were adapted to account for overdispersion and possible positional biases. Female wasps responded extremely well in tests where they were offered a single odour source, as well as in tests with multiple choices. The responses of wasps released in groups were the same as those released individually and it was found that females did not attract or repel each other, but males preferred arms in which females had been released. Dose–response tests with varying numbers of plants or host larvae on plants revealed that the wasps responded in a dose-related manner, thus showing that the system is well suited to measure relative preference. The clear choices of the insects amongst six possibilities provided substantial statistical power. Gas chromatographic analyses of sampled air revealed clean and effective odour trapping, which largely facilitates the comparison of results from behavioural assays with the actual blends of volatiles that were emitted by the various odour sources. Advantages and disadvantages compared to other methods are discussed.

Key words. *Cotesia marginiventris*, induced plant volatiles, log-linear model, maize, olfactometer, parasitoid behaviour, *Spodoptera littoralis*, *Zea mays*.

Introduction

Olfaction is the primary sense used by insects to detect and locate various resources (Whittaker & Feeny, 1971; Tumlinson *et al.*, 1993). Studies that aim to unravel odour-mediated interactions among insects and other organisms usually involve behavioural as well as chemical assays. These have resulted in the identification of numerous pheromone blends that insects use in sexual interactions (Mayer & McLaughlin, 1991; Cardé & Minks, 1996; Howse *et al.*, 1998), but have also revealed the sources and identities of substances that insects use to locate food and other resources (Bell & Cardé, 1984; Cardé & Bell, 1995). The studies are often tedious because of the time consuming behavioural assays that are involved. In the laboratory,

Correspondence: Dr Ted C. J. Turlings, University of Neuchâtel, Institute of Zoology, Laboratory of Animal Ecology and Entomology, C.P.2, CH-2007 Neuchâtel, Switzerland. Tel.: +41 32 718 3158; fax: +41 32 718 3001; e-mail: ted.turlings@unine.ch

insect responses are usually observed in olfactometers or flight tunnels of varying complexity (Hare, 1998).

Olfactometers have been used for over a century. One of the earliest and best descriptions of an olfactometer comes from McIndoo (1926); see also Snapp & Swingle (1929). He tested the attractiveness of host-plant odours to beetles by releasing the insects in the base of a Y-shaped glass tube where they were exposed to odours that were introduced through the two arms of the tube. An insect that walked into one of the arms was assumed to have a preference for the odour introduced through that arm. Y-Shaped olfactometers, or comparable T-shaped linear tract olfactometers (Sakuma & Fukami, 1985), are still commonly used to test the olfactory responses of various arthropods (Sabelis & vande Baan, 1983; Steinberg *et al.*, 1992; Bartlet *et al.*, 1997; Pallini *et al.*, 1997; Bernasconi *et al.*, 1998; Sullivan *et al.*, 2000).

Pettersson (1970) developed the four-arm olfactometer, which has been modified and described in more detail by Vet *et al.* (1983). In this olfactometer, small insects are introduced into a chamber in which four distinct odour fields are created. In principle, four different odour sources can be tested, but usually it is not used for more than two types of odours. Four-arm olfactometers are well suited for direct behavioural observations. Other types of olfactometers are mainly static, without airflow, but in which odour gradients determine the orientation of responding insects (Ruther & Steidle, 2000). Directional responses to odours by walking insects can be precisely recorded and measured with walking spheres, as developed by Thièry & Visser (1987).

For flying insects, flight tunnels can be employed (Miller & Roelofs, 1978; Baker & Linn, 1984). Because the insects will have to exhibit their full behavioural repertoire to reach an odour source, flight tunnel assays are often more sensitive to varying abiotic conditions, as well as the condition of the insects. In particular, the tendency for insects to fly towards light sources frequently prevents them from orienting towards odour sources. However, positive responses in flight tunnels provide the most convincing evidence that an odour source is truly attractive to an insect.

All these behavioural assays are very time consuming. They are usually designed for the release of one insect at a time, responding to a very limited number of odours. Moreover, the chemical identities of the substances that evoke the responses have to be determined in separate experiments. Recent developments in the odour trapping and sensitive analytical chemistry techniques have greatly facilitated such efforts (Millar & Haynes, 1998). In studies on the role of plant-provided cues in the host-location processes of parasitoids, several of these techniques have been employed (Turlings *et al.*, 1991b, 1998). However, the need to compare large numbers of odour sources for their relative attractiveness requires the development of novel assay techniques that allow for faster testing of multiple odour sources with larger numbers of insects.

The present study introduces an olfactometer in which six odours can be offered to test insects at the same time, and where part of each odour is collected on trapping filters for subsequent chemical analyses. Using naïve females of the parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), it is shown that this olfactometer can be highly effective in determining the relative preference for various odours. The wasps were strongly attracted to maize plants releasing an odour blend that is typical for plants under caterpillar attack. In dose–response tests, the wasps also clearly preferred sources that released more of these odours. By releasing wasps in groups, the experiments could be conducted in a relatively short time span. In separate experiments, it was shown that female wasps do not interfere with each other's behaviour, but that males are attracted to females.

Materials and methods

The insects

For all bioassays, 2–3-day-old naïve individuals of the solitary endoparasitoid *C. marginiventris* were used. The rearing colony originated from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi). For rearing, 25 *Spodoptera littoralis* (Noctuidae: Lepidoptera) caterpillars (3–4 days old) were offered to a single mated female (4–7 days old) for 3 h in a plastic-box (diameter 9.5 cm, height 5 cm). The parasitized caterpillars were kept on a wheatgerm-based artificial diet in an incubator (25 °C and LD 16 : 8 h) until cocoon formation. Cocoons were kept in Petri dishes until adult emergence. Emerging adults were sexed and kept in cages (30 × 30 × 30 cm) in the same incubator at a sex ratio of 1 : 2 (male : female), with moist cotton wool and honey as a food source.

Spodoptera littoralis larvae were reared from eggs provided by Syngenta (Stein, Switzerland). The eggs were kept in the above incubator and, after emergence, larvae were placed on the artificial diet at room temperature. In several experiments, second-instar larvae were directly placed on small maize plants to induce the production of volatiles. In other cases, caterpillar regurgitant was used for this induction. Regurgitant was collected from third- and fourth-instar larvae that had been fed on maize leaves, as described by Turlings *et al.* (1993). The collected material was centrifuged and the supernatant was filtered through a 0.22 μm filter to remove large particles and microorganisms, and subsequently stored at −70 °C until used for the treatment of plants.

The six-arm olfactometer

Details on the olfactometer are available at http://www.unine.ch/zool/leae/olfactometer.html. The device was assembled on a 1 × 1 m and 1.5 m high three-tiered metal frame on small wheels, with each level consisting of a wooden

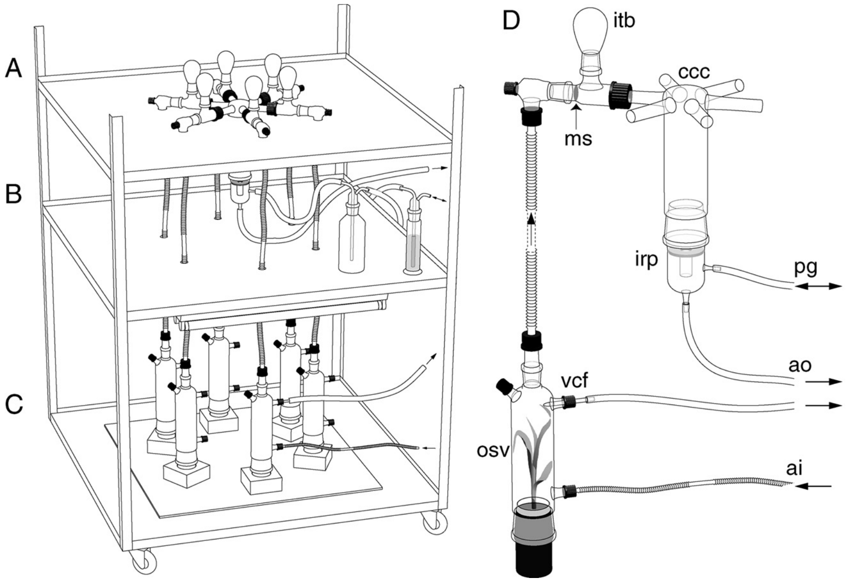


Fig. 1. An overview of the six-arm olfactometer. (A) Top shelf where the insects can make choice for one of six possible odours. (B) Middle shelf from where the insects are released. (C) Bottom shelf with the six odour sources (only for one of the chambers are all connecting tubes drawn). (D) Detailed depiction of the various parts and connections; ai, air inlet; osv, odour source vessel; vcf, volatile collection filter; ms, metal screen; ccc, central choice chamber; itb, insect trapping bulb; irp, insect release point; pg, pressure gauge; ao, air outlet. The corrugated tubes are made of Teflon and the exhaust tubes of Tygon (drawing by Dr Thomas Degen).

shelf (Fig. 1). Holes in the top two shelves allowed for the connection between the different levels via Teflon and glass tubing. The bottom shelf (10 cm from the floor) held the odour sources. A middle shelf (83 cm from the floor) served as a release point for the insects, whereas the top shelf (123 cm from the floor) carried the olfactometer itself. One of the main advantages of the separation of the different parts of the olfactometer is that each of them can be subjected to different light conditions. Moreover, the test insects have no visual contact with the odour sources. This separation was found to be practical and optimized the use of space. Almost all glass parts were custom made by Uni-Glas (Zurich, Switzerland) and VQT (Neuchâtel, Switzerland).

The air delivery

A central inhouse compressor supplied the air. Starting at the laboratory valve, air was first passed through two 16-cm long 1/2" Ø (external diameter) stainless steel tubes filled with 20–60 mesh activated charcoal (Sigma, St Louis, MO) for purification. The air then passed through a 1/4" Ø copper tube into a 1-L water bubbler that was half filled with deionized water. The pure and humid air was then pushed into a manifold with six flowmeters (Aalborg, Orangenburg, NY). In all experiments, the flow rate through each flowmeter was set at 1.2 L/min. The exit of each flowmeter was connected to a 13-cm long, 6-mm Ø glass tube filled with activated charcoal, held in place by two 325 mesh stainless steel screens (Small Parts Inc., Miami Lakes, Florida). These tubes served to filter out any remaining impurities. The glass tubes were connected to 1/4" Ø corrugated Teflon tubes (Cole-Parmer, Vernon Hills, Illinois) of sufficient (variable) length to reach one of the six odour source vessels.

The odour source vessels

The vertically-placed cylindrical odour source vessels (Fig.1) were designed to hold small living plants and consisted of three parts. The bottom part was an 11-cm high 5-cm internal diameter (i.d.) glass pot with a 50–55-mm male ground glass joint. This pot fitted the female ground glass joint of the 28.5-cm long, 6-cm i.d. central part of the vessel. Just above the female fitting, a horizontally connected glass port with a screw cap fitting (GL14) allowed for the connection to one of the Teflon air supply tubes through a rubber Teflon-coated 6-mm i.d. ferrule. The air was introduced via these tubes into each vessel, a few cm above the glass pot. Two identical glass ports were positioned at the other (top) end of the central part one perpendicular (horizontal) to the vessel and one under an angle pointing up. These ports served to hold trapping filters for volatile collections (Fig.1D). Just above these ports, the glass cylinder narrowed into a 22–25-mm ground glass connector. A fitting with a glass male joint and a screw-cap fitting (GL25) on the other end, formed the connection for a 3/4" corrugated Teflon tube that transfers the air from the odour source to one of the arms of the olfactometer on the top shelf. For this purpose, holes were drilled just above each vessel in the middle and top shelf.

Six such vessels, all connected to one of the Teflon tubes from the flowmeters, were placed in a 50-cm Ø circle at equal distances from each other. For stability, the bottom pots were placed in 4.5-cm high wooden blocks with a fitting hole in the centre that were glued to a wooden base (Fig. 1). They were illuminated with 10 neon tubes (five Osram 18 W/21-810, alternated with five Sylvania Gro-Lux F18W/GRO-T8) that were connected to the bottom of the second shelf of the olfactometer. These lamps provided approximately 8300 lux at plant height.

The olfactometer tiers

As shown in Fig. 1, the odour sources were connected to the olfactometer with corrugated Teflon tubing. The first connecting part was a glass elbow with a GL25 screw-cap fitting. This part ended in a 22–25-mm female ground glass connector and also had a GL14 screw-cap fitting, which was sealed during all experiments. The next glass part had a 22–25-mm male ground-glass connector and a similar upward connection for the insect-trapping bulb (50 mL) (Fig. 1D). A 325 mesh stainless metal screen (Small Parts Inc., Miami Lakes, FL) was placed just after the horizontal male connector and served to block the passage of insects that had made a choice. The opposite part was attached to one of the arms of the central choice chamber by another GL25 screw-cap fitting. This central chamber consisted of six 15-mm Ø arms attached to a 6-cm i.d., 22-cm long vertically placed cylinder into which the insects were released. The top of the cylinder was closed just above the arms and the rest was hanging through a hole in the top shelf. The base of the chamber was connected via a 50–55-mm ground-glass joint to a shorter cylinder with a glass frit into which a 15-mm Ø insect release vial was sealed (Fig. 1D). The bottom of this cylinder tapered off into a 6-mm tube connector and a similar connector was attached to the side. The first was connected with a Tygon tube to the vacuum pump via another flowmeter, whereas the second connection was with a wash bottle with water that served as a pressure gauge. Both were used to balance the incoming and outgoing air (6 × 600 mL/h).

To eliminate any visual distractions and to ensure uniform illumination, the olfactometer on the top tier was surrounded by a 56-cm Ø, 25-cm-high white cardboard cylinder (not shown in Fig. 1). A second such cylinder (23-cm Ø, 25-cm high) with holes cut out for the olfactometer arms was placed just around the central choice chamber and a milky white Plexiglas disk (28-cm Ø, 3-mm thick) was placed on top of this cylinder. This disk was illuminated with a 60 W light bulb placed 25 cm above it. Because the inner-cylinder was covered, the central choice chamber was not visible to the experimenter, but the insect trapping bulbs were positioned outside of this cylinder and could be readily checked for the presence of insects by looking into the larger cylinder.

Bioassays

Mated 2–3-day-old female (in one case male) wasps were used and all test insects were naïve in that they had never encountered a host or plant as an adult. In most cases, the wasps were released in groups of six (exceptions are specified below). They were removed from their cage with an aspirator and directly placed in the vial attached to a glass frit at the bottom of the central choice chamber (the insect release point in Fig. 1D). The great majority of the wasps walked up, attracted by the light above the chamber. If attractive odours were present, most of the wasps would walk into an arm with such an odour until their path was blocked by the stainless steel screen. Eventually, they walked up in the direction of the light source into the insect trapping bulb (Fig. 1D), where they could easily be counted and removed. All insects were given 30 min to make a choice, after which they all were removed and a new group was released. Three groups of six wasps were tested on a given day. Statistical comparisons among choices are described in the results section.

On a given day, all wasps were tested with the same odour sources, which remained in the same position. This was performed because preliminary results had shown that the arms remained attractive after removal of attractive sources due to adsorption of volatiles to glass and Teflon surfaces. For this reason, all glass and Teflon parts were carefully cleaned at the end of an experiment (day), first with water and then by rinsing with acetone and pentane. After the solvents had evaporated, the glass parts were placed overnight in an oven at 250 °C.

Odour sources

In almost all cases, 8–9-day-old maize plants (var. Delprim) served as odour sources. They were individually grown from seed placed in plastic pots (6-cm high, 8-cm diameter) in fertilized commercial soil (Coop, Switzerland). The pots were placed in a climate chamber (23 °C, 60% RH, and LD 16 : 8 h, 50000 lux) and, the day before an experiment, the plants were transplanted into a glass pot with a ground-glass male connector that could be inserted into an odour source vessel. Plants were induced to emit volatiles by either placing a certain number of second-instar *Spodoptera littoralis* larvae on them on the evening before an experimental day, by scratching two leaves (2-cm^2) and applying 10 µL *S. littoralis* regurgitant to the damaged sites (also on the evening before). In an experiment to test for intraspecific interference, female parasitoids were used as potential odour sources, as detailed below.

Odour trapping

Volatiles emitted by the various odour sources in the above-described vessels were trapped in a similar manner to lings *et al.* (1998). Trapping filters consisted of 7-cm glass tubes in which 25 mg of 80–100 mesh Super Q adsorbent (Altech, Deerfield, Illinois) was placed and kept in place by two fine mesh metal screens, as described by Heath & Manukian (1992). In all experiments, one filter was attached to the horizontal port at the top of each odour source vessel (Fig. 1D). The other ports were sealed with a Teflon-coated septum in the screw cap. A 6-mm i.d. Tygon tube was connected to each collection trap. These tubes were attached to a second manifold with six flowmeters (Aalborg Instruments & Controls Inc., Monsey, New York). Air was pulled through each tube at a rate of 0.6 L/min (half of the incoming flow) via additional Tygon tubing connecting the flowmeters to a vacuum pump (model ME2, Vacuubrand, Germany). The vacuum was turned on 1 min after the air was first pushed into the system.

Before each experiment, the traps were rinsed five times with 200 μL methylene chloride. Collections always lasted 3 h. The first olfactometer tests started 30 min after a collection began. After each collection, the traps were removed, extracted and analysed as described below.

Chemical analysis

During all experiments 50% of the air passing over the odour sources was pulled through a trapping filter for 3 h (see above). Immediately after each experiment, the volatiles collected on these filters were extracted with 150 μL of methylene chloride and two internal standards (*n*-octane and nonyl acetate, each 200 ng in 10 μL methylene chloride) were added to these samples. The samples were either analysed immediately or stored at −70 °C before analysis. For the analysis of each sample, an aliquot of 3 μL was injected on column with the use of an automated injection system onto an apolar HP-1 capillary column (30 m, 0.25-mm i.d., 0.25 μm film thickness). The column was housed in a Hewlett Packard model HP 6890 gas chromatograph equipped with a flame ionization detector. The oven was held at 50 °C for 3 min and then programmed at 8 °C/min to 230 °C, where it was maintained for 9.5 min. The column was preceded by a deactivated retention gap (10 m, 0.25-mm i.d., Connex) and a deactivated precolumn (30-cm, 0.53-mm i.d., Connex). Helium (24 cm/s) was used as carrier gas. HP GC Chemstation software was used to quantify all major components based on the known quantity of internal standards. Initial identification of most compounds was based on comparisons of retention times from previous studies (Bernasconi *et al.*, 1998; Turlings *et al.*, 1998). Identities were confirmed with the mass spectrometry analysis of some samples, using the same column and temperature programme (Agilent 5973, transfer line 230 °C, source 230 °C, quadrupole 150 °C, ionization potential 70 eV, scan range 0–400 amu). Total quantities of volatiles were calculated based on their peak areas compared to those of the internal standards.

Statistical analysis

Except for the first experiment, the results for each test day were used as a replication (a minimum of six replicates). An initial experiment designed to test for a directional bias of the wasps found none but, nonetheless, bias was tested in each subsequent experiment; none was found. Only those insects that made a choice were included in the analyses of the results; these represented over 75% of the wasps in experiments where an attraction to an odour source was expected. Thus the responses consist of counts ($n_1, \ldots, n_6$), where n_i denotes the number of wasps observed to choose arm *i*. Simple data analyses would suppose that the counts ($n_1, \ldots, n_6$) for a single replicate follow a multinomial distribution with probabilities ($P_1, \ldots, P_6$) and denominator m the total number of wasps making a choice; here P_i represents the probability that an individual wasp chooses arm *i*. Such a model accounts automatically for the dependence between the numbers of wasps choosing the different arms. Its underlying assumptions are that individual wasps act entirely independently of one another and that the P_i are constant across all replicates with the same experimental conditions. If these assumptions are true, it is possible to model how the probabilities $P_1, \ldots, P_6$ depend on the experimental treatments. For example, in the dose–response experiments, a log-linear form $\log P_i = b_0 + b_1 x_i - c$ was used, whereby the log probability of choice of arm *i* is taken to depend linearly on a covariate x_i, which measures the relative attractiveness of the dose for that arm; Model I. The constant c ensures that $P_1 + \ldots + P_6 = 1$; it depends on the unknown parameters b_0 and b_1, which must be estimated from the data. The strength of the attraction is measured by b_1; if this is close to zero, then the attraction is weak, whereas a large positive value indicates strong attraction. If such a model is supported by the data, a test of attraction may be based on the estimate of b_1. Log-linear models such as this are widely used in applied science and their statistical properties are well-understood (Bishop *et al.* 1975; Dobson, 1989; Davison, 2003). They are readily adapted to more complex experimental setups. The interspecific interference experiments described below use a 2 × 2 factorial design, for example, with arms 1, 3, 5 given no treatment, and the other three treated with plant odour (e.g. arm 2), 10 female insects (e.g. arm 4) and both plant odour and 10 female insects (e.g. arm 6). The corresponding log-linear model (Model II) sets $\log P_1 = \log P_3 = \log P_5 = -c$, $\log P_2 = g_1 - c$, $\log P_4 = g_2 - c$, and $\log P_6 = g_1 + g_2 + g_3 - c$, where c ensures that the total probability is one, g_1 represents the relative attraction of plant odour, g_2 the relative attractiveness of female wasps, and g_3 is an interaction between these. Significantly positive estimates of g_1 or g_2 would indicate significant attraction of the corresponding treatments for the wasps, and the sizes of these estimates measure the relative strength of attraction. Such models can be fitted by maximum likelihood estimation in software packages such as *R* (http://stat.ethz.ch/CRAN/), and their relative adequacy can be assessed through likelihood ratio statistics and examination of

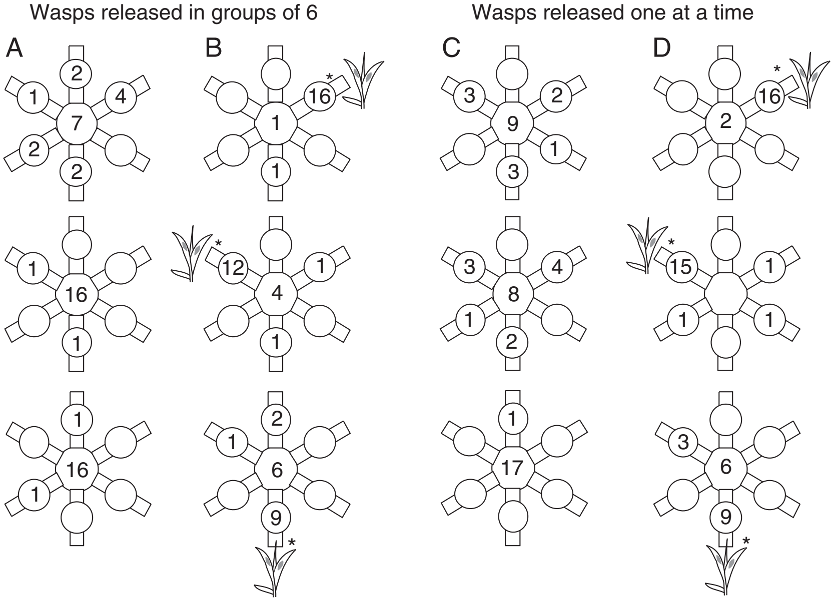


Fig. 2. Results for the experiments with no (A and C) or a single (B and D) odour source. Each drawing represents a single replicate during which 18 female wasps were released. The wasps were released either singly or in groups of six. Each replicate was tested separately for differences in the number of choices among the arms. Arms with an asterisk were chosen significantly more often than the other five arms.

residuals. More details for the data are provided in the results section.

A complication for the use of both standard tests and modelling is overdispersion, the statistical term for situations in which data vary by more than would be expected under a theoretical model such as the multinomial. This often arises in applications because the assumptions that underlie simple statistical models are rarely entirely valid. Overdispersion is present in the data, perhaps owing to the slight variation in experimental conditions between and within replicates. Fortunately, its effects are well studied and there are standard techniques for assessing the degree of overdispersion and obtaining reliable inferences from overdispersed data (Firth, 1991; McCullagh & Nelder, 1989; Davison, 2003). Modified *G*-statistics were used for comparison of log-linear models fitted to overdispersed data, and modified standard errors for parameter estimates.

Results

Blank tests and single choice experiments

In a first experiment, the responses of naïve *Cotesia marginiventris* females were tested with no odour sources placed in any of the chambers. This experiment was conducted to test for directional biases of the insects and to determine if the wasps would enter the arms in the absence of an odour. Female wasps (2–4 days old) were released either individually or in groups of six and left in the olfactometer for 30 min or until they had made a choice. In both cases, the majority of the wasps remained in the central chamber of the olfactometer. The few wasps that did make a choice showed no significant preference for any of the arms (Figs 2A,C) ($P > 0.05$ in both cases). There was evidence of different behaviour in the three different replicates of the experiment ($P < 0.01$ in both cases), though this is based on the small numbers of wasps that made choices. However, such variation between replicates performed under supposedly identical conditions may account for the overdispersion mentioned in the section on statistical treatment of the data, and for which allowance is made below.

In a subsequent experiment, a mechanically damaged maize plant treated with regurgitant (see above) served as an odour source in one of the vessels. All other odour source vessels were empty. Again, wasps were either released individually or in groups of six. The position of the plant was different for each of the three replicates (Figs 2B,D). Of the 18 females that were released per replicate, the great majority chose the arm with the maize odour (Figs 2B,D). These arms were chosen significantly more often than arms with no odour ($P < 0.001$ in both cases).

Collected odour emissions

During all assays, the volatiles were trapped by pulling 50% of the airflow through a filter that was attached to the top of each odour source vessel. Figure 3 shows two typical chromatograms; one of volatiles collected from an empty vessel and one of volatiles collected from a maize plant treated with caterpillar regurgitant. The blank collection reveals very small amounts of a few impurities in the system, whereas the collection of the plant odour

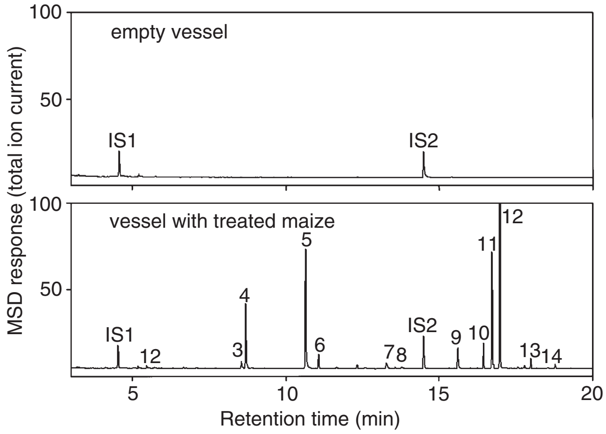


Fig. 3. Typical chromatograms of collected volatiles from either an empty odour source vessel (blank) or a maize plant that was treated with the regurgitant of *Spodoptera* larvae (treated plant). The labelled peaks represent: (1) (*E*)-2-hexenal; (2) (*Z*)-3-hexenol; (3) β-myrcene; (4) (*Z*)-3-hexen-1-yl acetate; (5) linalool; (6) (3*E*)-4,8-dimethyl-1,3,7-nonatriene; (7) phenethyl acetate; (8) indole; (9) geranyl acetate; (10) β-caryophyllene; (11) (*E*)-α-bergamotene; (12) *E*-β-farnesene; (13) unknown sesquiterpene; and (14) (3*E*, 7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

demonstrates that all typical induced maize volatiles (Turlings *et al.*, 1998) were emitted and readily collected (Fig. 3).

Intraspecific interference

To test the possibility that the wasps affect each other's choices by either attracting or repelling one another, two experiments were conducted with females already present in one or two of the arms. In the first experiment, 10 females were placed in one of the arms for 1 h when plugs of cotton were used to prevent them from either walking up into the trapping bulb or out of the arm into the choice chamber. By this method, any odour emitted by the females could adsorb onto the glass in the arm. After 1 h, the cotton plugs were removed and the normal experimental airflows were passed through all arms. The wasps were left in the arm and were given 30 min to settle in the trapping bulb before starting the actual assays. The five other arms were left empty.

On each test day, three groups of six females and three groups of six males were released in the choice chamber and their choices were recorded. This was repeated for 6 days, each time with the 10 females in a different arm. Most females did not enter an arm and those that did distributed themselves evenly over all six arms (Fig. 4A). On the other hand, the males entered the arm with females significantly more than the control arms (Fig. 4B) ($P < 0.001$).

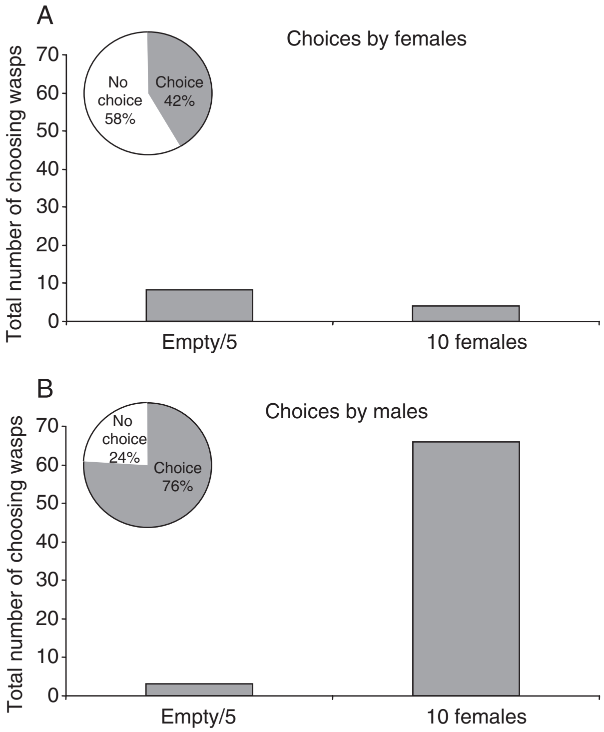


Fig. 4. Responses to the odour of female conspecifics, which was offered through only one of the six arms. The pie chart shows the percentage of wasps that entered an arm. Choices for the control arms were summed and divided by 5. (A) Choices made by females. (B) Choices made by males.

In the second experiment, three odours were introduced that were alternated with arms with clean air only. For two of the odours, two treated maize plants were placed in the bottom vessel. In one case, this was combined with 10 females that were placed in the arm as described above. Again, after 1 h, the cotton plugs that kept them from moving out were removed and the normal experimental airflows were passed through all arms. The third odour arm received 10 females in the same way, but did not have the plant odour.

Again on each test day, three groups of six females were released in the choice chamber and their choices were recorded. This time no males were tested. This was repeated for 6 days, with the different odour being introduced through different arms. The great majority of females chose arms with plant odour and did not distinguish between arms that already contained other wasps and those that did not (Fig. 5). A fit of Model II to these data by maximum likelihood estimation with allowance for over-dispersion led to estimates (standard errors) for g_1 (attraction of plant), g_2 (attraction of females) and g_3 (interaction between plant and females) of 3.05 (0.58), −8.20 (34.7) and 8.10 (34.7), with respective two-sided significance levels of 10^{-5}, 0.8 and 0.8; without allowance for overdispersion the significance level for g_1 would have been 10^{-12}, thus showing how failure to account for overdispersion might give misleading conclusions. This confirms the visual impression from Fig. 5. The attraction of plants was highly significant and the presence of other females did not affect this attraction.

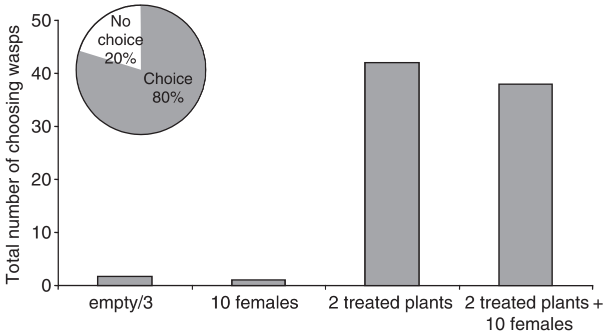


Fig. 5. Responses to the odour of female conspecifics in the presence or absence of induced maize odour. The pie chart shows the percentage of wasps that entered an arm. Choices for the three control arms were summed and divided by 3.

Dose–response tests

Two dose–response tests were conducted to test if the olfactometer was suited to measure relative attractiveness of multiple odours. In a first experiment, three arms with only clean air were alternated with arms with the odour of either one, two or three maize plants that were damaged and treated with regurgitant as described above. Three times, six naïve wasps were released per replicate (six replicates) and their choices were determined 30 min after release. For each replicate, the odour sources were placed in a different position, but always with one empty chamber between two chambers that contained at least one plant. The wasps responded in a dose-related manner (Fig. 6A), with most of the wasps choosing the arm with three plants. The analyses of the volatiles that were collected during the bioassays confirmed that the total amount emitted was positively correlated with the number of plants per odour sources, although fits of Model I showed that the numbers of wasps choosing an arm was more highly correlated with the number of plants than with the amount emitted (significance levels of 0.00005 and 0.004, respectively). There is very strong evidence that the number of wasps choosing an arm increases both with the number of plants and the volatiles emitted.

For the second dose–response experiment, two maize plants were placed in each of the six chambers, but they received different numbers (0, 2, 4, 8, 16 or 32) of second-instar *Spodoptera littoralis* larvae. The larvae were placed on the plants at 17.00 h the day before the assays. The next morning, three groups of six naïve female wasps were released in the olfactometer. Again, the choices for the arms were dose-related, with the majority ending up in the arm that carried the odour of plants damaged by 32 larvae (Fig. 6B). The more larvae that were on the two plants, the more odour was emitted (Fig. 6B). To obtain a numerical measure of the strength of the attraction of the odour from damaged plants, Model I was fitted, with x_i taken as the base 10 logarithm of total amount of collected volatiles (in ng), rescaled so that the lowest value of x_i for each experiment was zero; thus x_i represents the relative strength of the odour for each experiment. The fit gave an estimate (standard error) for b_1 of 1.27 (0.29), with a significance level of around 0.0001. This shows a strong correlation between odour intensity and attraction. The results of both experiments show that the wasps are able to distinguish among odours in the olfactometer and that they make their choices accordingly.

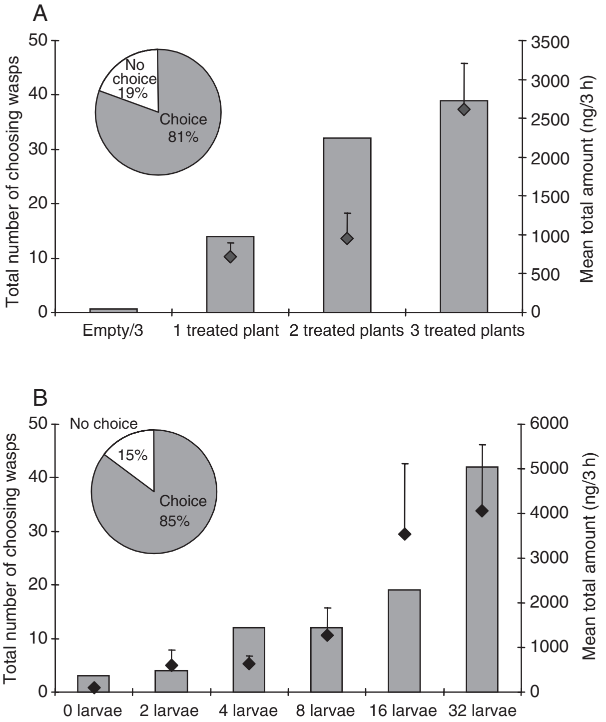


Fig. 6. Dose–response tests. The pie charts show the percentage of wasps that entered an arm. The bars indicate the choices made for the different arms, whereas black diamond symbols indicate the total amounts of volatiles emitted by an odour source. (A) Arms with one, two or three induced maize plants were alternated with empty arms. Choices for the three empty arms were summed and divided by 3. (B) Odour sources were two maize plants with 0, 2, 4, 8, 16 or 32 *Spodoptera* larvae.

Discussion

Females of the larval endoparasitoid *Cotesia marginiventris* respond exceptionally well in the six-arm olfactometer. In previous olfactometric studies, female wasps required conditioning with an oviposition experience in the presence of a particular odour source in order for them to respond to odours in a four-arm olfactometer or a flight tunnel (Turlings *et al.*, 1989, 1991a). Here, a great majority of unconditioned (naïve) females make a choice for an arm if the odour introduced through that arm is from a treated maize plant. If no such odour is offered, most females remain in the central release chamber during the 30-min test period (Fig. 2). Similar results are being obtained with several other parasitoid species.

The results confirm previous findings that *C. marginiventris* is attracted to herbivore-damaged maize plants (Turlings *et al.*, 1990; Turlings *et al.*, 1995). This is also clearly the case for naïve insects that have not had contact with a host and never experienced the odour of the plant before. Nearly 90% of the females choose an arm with the odour of a treated plant when it is offered next to five arms that did not carry an odour (Fig. 2).

Experimental design and interpretation of the results

Ramirez *et al.* (2000) expressed various concerns about the design of published olfactometer and wind tunnel studies. Pseudo-replication and a lack of independence are very common flaws of many studies. The design of the current experiments varies because of differences in the purpose of each test. In the first series of experiments, the principal goal was to determine whether the olfactometer can be used to test the attractiveness of an odour. For this reason, a single odour source (damaged maize plants treated with caterpillar regurgitant) is used that is known to be attractive to the wasps (Turlings *et al.*, 1990). Given that a great majority of the females enter the arm carrying the odour of the plant (Fig. 2), the olfactometer is suited to test the attractiveness of individual odours. By placing the plant in a different chamber for different replicates, the possibility that the results are due to a positional effect is ruled out. This is also confirmed with the experiments in which none of the chambers contain a plant; most females stay in the central chamber and those that do walk into an arm do not show a significant preference for a particular arm.

This olfactometer was specifically designed for 'high-throughput' studies, whereby insects are released in groups. Therefore, a second objective of the first experiments was to test if groups of females can be released at the same time without influencing each other's choices. This is clearly the case because the results for single releases are almost identical to those for group releases (Fig. 2). It is also shown that females are not attracted or repelled by each other (Fig. 4) and *C. marginiventris* females released in groups can be assumed to make independent choices. This should be determined anew for each insect species that is tested in the olfactometer. Interestingly, male *C. marginiventris* are attracted to arms with females, probably due to the presence of a sex pheromone.

The aim of the dose–response experiments was to determine if the olfactometer can be used to test the relative attractiveness of simultaneously offered odours. For such experiments, an appropriate replication is of the utmost importance. The unit of replication in the first place is the odour source and, in the second place, the insect. Ideally, each insect is exposed to a new combination of odours. This is entirely impractical, but to avoid false conclusions due to pseudo-replication, the experiments need to be replicated sufficiently with an entirely new set of odour sources. Here, each experiment with multiple odour sources is replicated six times and the position of the odour sources is randomized (for the experiment with different number of larvae) or covers all possible positions of the odours (for the experiment with different numbers of plants).

The results for the dose–response tests (Fig. 5) illustrate that the insects make clear choices. Arms carrying the odour of just one treated plant are chosen less often than arms with two or three plants. Similarly, the wasps respond in a dose-related manner to plants that are fed upon by different numbers of *Spodoptera littoralis* larvae, with the highest number of wasps choosing the plants with the most (32) larvae. In both dose–response tests, the amount of volatiles collected during the bioassays corresponds with the preferences the females exhibited.

Statistics

The choice of appropriate statistics for the evaluation of the results of olfactometer studies is not always straightforward. In cases where there are only two choices, several simple tests are available but, for multiple choices, various tests have been employed. Standard analysis of variance is not appropriate because the responses are categorical rather than numerical measures. Standard log-linear models for categorical data can be employed, but it is important to adjust their output to account for the overdispersion commonly present with such data. Failure to make such adjustment could result in erroneously small *P*-values and standard errors, resulting in statements that certain effects are present, when in fact the data do not justify them. If present, overdispersion also invalidates standard approaches such as the use of tests of homogeneity based on G-statistics (Scherrer, 1984; Sokal & Rohlf, 1995).

Advantages and disadvantages

With the use of this six-arm olfactometer, multiple odours can be tested at the same time and insects can be released in groups, which constitutes a major advantage, saving experimental and rearing time. Moreover, when only one or few odours are tested, the six-arm olfactometer offers more statistical power than other olfactometric devices with fewer choices. This power of statistics stems from the fact that the likelihood of an insect selecting an arm with the test odour by chance is much smaller than in a two-choice situation.

Another important advantage is the simultaneous trapping of volatiles during the behavioural assays. The subsequent analysis of the volatiles allows for a direct comparison of the assay results with the actual quantity and quality of the odour that the insects responds to. Variation in odour emissions among similar treatment can therefore be accounted for. The volatile collections contain surprisingly few impurities despite the fact that the plants are standing in regular potting soil. All major induced maize volatiles (Turlings *et al.*, 1998) are readily detected in the analyses.

The separation of odour sources and insects by using different shelves ensures that the insects have no visual cues to orientate by. Moreover, it is possible to use different illumination for insects and plants. A central moderate light source above the olfactometer is used to initially attract the wasps up into the choice chamber, whereas the plants that serve as an odour source are exposed to considerably more light to ensure good odour emissions. The release of induced odour by maize plants requires light and increases with increased light intensity (Gouinguene & Turlings, 2002).

Disadvantages of this device are the relatively high costs for construction and the vulnerability of its many glass parts to damage. Most inconvenient is the need to clean the entire device between each replicate. Preliminary tests had shown that adsorption of the attractive odours on glass and/or Teflon parts rendered arms attractive even after an odour source is removed. This is the reason why the odour sources are only tested in one particular position on a given day. Despite these disadvantages, the six-arm olfactometer is highly efficient and practical, and promises to be a useful tool for many types of olfactometric studies.

Acknowledgements

We thank Thomas Degen, Michael Rostás and Martine Rahier for fruitful discussions and/or useful comments on the manuscript. We are grateful to Thomas Degen for the drawing of the olfactometer. This work was supported by the Swiss National Science Foundation (grants 31-46237-95 and 31-058865-99) and was a collaborative effort conducted in the context of the Swiss National Centre of Competence in Research 'Plant Survival'.

References

Baker, T.C. & Linn, C.E. (1984) Wind tunnels in pheromone research. *Techniques in Pheromone Research* (ed. by T. A. Miller), pp. 74–110. Springer Verlag, New York.

Bartlet, E., Blight, M.M., Lane, P. & Williams, I.H. (1997) The responses of the cabbage seed weevil *Ceutorhynchus assimilis* to volatile compounds from oilseed rape in a linear track olfactometer. *Entomologia Experimentalis et Applicata*, **85**, 257–262.

Bell, W.J. & Cardé, R.T. (1984) *Chemical Ecology of Insects*. Chapman & Hall, London.

Bernasconi, M.L., Turlings, T.C.J., Ambrosetti, L., Bassetti, P. & Dorn, S. (1998) Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomologia Experimentalis et Applicata*, **87**, 133–142.

Bishop, Y.M., Fienberg, S.E. & Holland, P.W. (1975) *Discrete Multivariate Analysis*. MIT Press, Cambridge, MA.

Cardé, R.T. & Bell, A.A. (1995) *Chemical Ecology of Insects 2*. Chapman & Hall, New York.

Cardé, R.T. & Minks, A.K. (1996) *Insect Pheromone Research. New Directions*. Chapman & Hall, New York.

Davison, A.C. (2003) *Statistical Models*. Cambridge University Press, Cambridge.

Dobson, A.J. (1989) *An Introduction to Generalized Linear Models*. Chapman & Hall, New York.

Firth, D. (1991) Generalized linear models. *Statistical Theory and Modelling* (ed. by D. V. Hinkley, N. Reid and E. J. Snell), pp. 55–82. Chapman & Hall, London.

Gouinguené, S.P. & Turlings, T.C.J. (2002) The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, **129**, 1296–1307.

Hare, J.D. (1998) Bioassays with terrestrial invertebrates. *Methods in Chemical Ecology, Bioassays Methods* (ed. by K. F. Hayes), vol. 2, pp. 212–270. Kluwer Academic, Norwell, MA.

Heath, R.R. & Manukian, A. (1992) Development and evaluation of systems to collect volatile semiochemicals from insects and plants using a charcoal-infused medium for air purification. *Journal of Chemical Ecology*, **18**, 1209–1226.

Howse, P.E., Stevens, I.D.R. & Jones, O.T. (1998) *Insect Pheromones and Their Use in Pest Management*. Chapman & Hall, London.

Mayer, M.S. & McLaughlin, J.R. (1991) *Handbook of Insects Pheromones and Sex Attractants*. CRC Press, Boca Raton, Florida.

McCullagh, P. & Nelder, J.A. (1989) *Generalized Linear Models*. Chapman & Hall, London.

McIndoo, N.E. (1926) An insect olfactometer. *Journal of Economic Entomology*, **19**, 545–571.

Millar, J.G. & Haynes, K.F. (1998) *Methods in Chemical Ecology 1*. Chapman & Hall, New York.

Miller, J.R. & Roelofs, W.L. (1978) Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. *Journal of Chemical Ecology*, **4**, 685–699.

Pallini, A., Janssen, A. & Sabelis, M.W. (1997) Odour-mediated responses of phytophagous mites to conspecific and heterospecific competitors. *Oecologia*, **110**, 179–185.

Pettersson, J. (1970) An aphid sex attractant. I. Biological studies. *Entomologica Scandinavica*, **1**, 63–73.

Ramirez, C.C., Fuentes-Contreras, E., Rodriguez, L.C. & Niemeyer, H.M. (2000) Pseudoreplication and its frequency in olfactometric laboratory studies. *Journal of Chemical Ecology*, **26**, 1423–1431.

Ruther, J. & Steidle, J.L.M. (2000) Mites as matchmakers: semiochemicals from host-associated mites attract both sexes of the parasitoid *Lariophagus distinguendus*. *Journal of Chemical Ecology*, **26**, 1205–1217.

Sabelis, M.W. & vande Baan, H.E. (1983) Location of distant spider mite colonies by Phytoseiid predators – demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. *Entomologia Experimentalis et Applicata*, **33**, 303–314.

Sakuma, M. & Fukami, H. (1985) The linear track olfactometer – an assay device for taxes of the german cockroach, *Blattella germanica* (L) (Dictyoptera, Blattellidae) toward their aggregation pheromone. *Applied Entomology and Zoology*, **20**, 387–402.

Scherrer, B. (1984) *Biostatistique*. Gaîtan Morin, Chicoutimi, Canada.

Snapp, O.I. & Swingle, H.S. (1929) Further results with the McIndoo olfactometer. *Journal of Economic Entomology*, **22**, 984–985.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. WH Freeman, New York.

Steinberg, S., Dicke, M., Vet, L.E.M. & Wanningen, R. (1992) Response of the braconid parasitoid *Cotesia* (= *Apanteles*) *glomerata* to volatile infochemicals – effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomologia Experimentalis et Applicata*, **63**, 163–175.

Sullivan, B.T., Pettersson, E.M., Seltmann, K.C. & Berisford, C.W. (2000) Attraction of the bark beetle parasitoid *Roptrocerus xylophagorum* (Hymenoptera: Pteromalidae) to host-associated olfactory cues. *Environmental Entomology*, **29**, 1138–1151.

Thièry, D. & Visser, J.H. (1987) Misleading the Colorado beetle with an odor blend. *Journal of Chemical Ecology*, **13**, 1139–1146.

Tumlinson, J.H., Turlings, T.C.J. & Lewis, W.J. (1993) Semiochemically mediated foraging behavior in beneficial parasitic insects. *Archives of Insect Biochemistry and Physiology*, **22**, 385–391.

Turlings, T.C.J., Tumlinson, J.H., Lewis, W.J. & Vet, L.E.M. (1989) Beneficial arthropod behavior mediated by airborne semiochemicals. 8. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *Journal of Insect Behavior*, **2**, 217–225.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, **250**, 1251–1253.

Turlings, T.C.J., McCall, P.J., Alborn, H.T. & Tumlinson, J.H. (1993) An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology*, **19**, 411–425.

Turlings, T.C.J., Loughrin, J.H., Mccall, P.J., Rose, U.S.R., Lewis, W.J. & Tumlinson, J.H. (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 4169–4174.

Turlings, T.C.J., Lengwiler, U.B., Bernasconi, M.L. & Wechsler, D. (1998) Timing of induced volatile emissions in maize seedlings. *Planta*, **207**, 146–152.

Turlings, T.C.J., Tumlinson, J.H., Eller, F.J. & Lewis, W.J. (1991a) Larval-damaged plants – source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. *Entomologia Experimentalis et Applicata*, **58**, 75–82.

Turlings, T.C.J., Tumlinson, J.H., Heath, R.R., Proveaux, A.T. & Doolittle, R.E. (1991b) Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. *Journal of Chemical Ecology*, **17**, 2235–2251.

Vet, L.E.M., van Lenteren, J.C., Heymans, M. & Meelis, E. (1983) An air-flow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiological Entomology*, **8**, 97–106.

Whittaker, R.H. & Feeny, P.P. (1971) Allelochemics – chemical interactions between species. *Science*, **171**, 757–770.

Accepted 20 October 2003

Chapter 2

The role of chemical signals in mate finding by the parasitoids *Cotesia marginiventris* and *Microplitis rufiventris*

Based on: Cristina Tamò and Ted C. J. Turlings· The role of chemical signals in mate finding by the parasitoids *Cotesia marginiventris* and *Microplitis rufiventris*. In preparation for submission to *Journal of Insect Behavior*.

Abstract

Using a six-arm olfactometer, we first investigated the chemical-mediated long-range attraction between males and females of the two parasitoids *Cotesia marginiventris* and *Microplitis rufiventris*. Males of both species were attracted to the odor of their conspecific females, but males were not attracted to other males, and females were not attracted to either sex. In a second series of experiments, we found that, unlike females, the male parasitoids were not attracted to volatiles emitted by plants under attack by caterpillar hosts, also not after experiencing the odor during mating. Hence, males of both species respond to female-produced pheromones, but they do not seem to make use of plant volatiles for mate finding and do not use learning as a means to associate potential mates with an experienced odor.

Introduction

Males of various different parasitoids species are attracted to females from a certain distance because of female-produced volatile sex pheromones (Lewis *et al.*, 1971; Vinson, 1972; Mohamed and Coppel, 1987; Field and Keller, 1993; Reed *et al.*, 1994; McNeil and Brodeur, 1995; Fauvergue *et al.*, 1999; Rungrojwanich and Walter, 2000; Sullivan, 2002;), for reviews see Eller *et al.* (1984) and Fauvergue *et al.* (1999). However, the isolation and identification of sex-pheromones in parasitoids has been reported for only few species (Robacker and Hendry, 1977; Eller *et al.*, 1984; Kainoh *et al.*, 1991; Swedenborg *et al.*, 1994; DeLury *et al.*, 1999). Even fewer studies have investigated the possible attraction of female parasitoids to conspecifics of either sex, or the attraction of males to conspecific males. One such study was carried out by Kimani and Overholt (1995). Using a Y-tube olfactometer they found that both males and females of *Cotesia flavipes* perceived and responded to odors emitted by the opposite sex. In another study, where both sexes were tested, males of the egg-parasitoid

Ascogaster quadridentata were found to be attracted to the extracts of females in a Y-tube olfactometer, but not to the extract of males, whereas females where not attracted to the extracts of males (De Lury *et al.*, 1999). For the larval parasitoid *Macrocentris grandii* it has been found that males are attracted to the odors extracts of both males and females, but females do not respond to either extract (Swedenborg and Jones, 1992). In some other parasitoid species males form swarms that are attractive to females (van den Assem, 1986).

For some herbivorous insect species it is known that they may use plant volatiles in addition to sex pheromones as mate finding cues e.g. (Landolt *et al.*, 1994; Landolt and Phillips, 1997; Reinecke *et al.*, 2005). For parasitoids and other natural enemies of herbivores the role of plant odors in mating finding has rarely been studied, while an increasing number of studies report on the key role of plant volatiles in host finding by female parasitoids (Whitman, 1988; Vet and Dicke, 1992; Turlings and Benrey, 1998; Turlings and Wäckers, 2004). In behavioral studies males usually do not respond to plant cues (Eller *et al.*, 1988; Messing and Jang, 1992; Udayagiri and Jones, 1992; Jones, 1996; Pettersson *et al.*, 2000; Pettersson, 2001). However, measurements of the olfactory sensitivity with electroantennogram technique (EAG) show that male and female antennae are equally sensitive to typical plant volatiles (Lecomte and Pouzat, 1985; Ramachandran and Norris, 1991; Salom *et al.*, 1991; Li *et al.*, 1992; Ochieng *et al.*, 2000; Pettersson *et al.*, 2000; Park *et al.*, 2001). Hence, males and females carry similar chemoreceptors and males can be expected to respond to plant odors under certain circumstances, perhaps in combination with other cues to locate mates. Some studies provide evidence for such a role for plant volatiles. *Cotesia plutella* males are attracted to host-damaged cabbage plants, suggesting that males of this species make use of plant volatiles to find females (Kawaguchi and Tanaka, 1999). It has also been reported that males of the ichneumonid parasitoid *Campoletis sonorensis* have a higher likelihood of successful mate finding in the presence of cotton plants (McAuslane *et al.*, 1990), possibly a consequence of responses to cotton odors. It remains unclear, however, how common it is that male parasitoids respond to plant odors and whether they can learn to use these cues to find mate, in the same way females do to find host (Turlings *et al.*, 1993; *Vet et al.*, 1995).

In the current study we tested the role of odors in mate finding by *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) and *Microplitis rufiventris* (Kokujev) (Hymenoptera: Braconidae), two solitary generalist larval braconid endoparasitoids that attack a broad range of lepidopteran hosts, many of which are important crop pests. Finding mates can be expected to be difficult for solitary parasitoids like *C. marginiventris* and *M. rufiventris*, especially when they occur at low densities, because mates may not be available at emergences sites. For these reasons, we expect that such species have developed a communication system, which allows mates to find each other over a certain distance. Therefore, using a six-arm olfactometer, we first carried out a series of experiments to test the attraction of males and females of these two species to conspecifics of the opposite or same sex.

In a second series of experiments we investigate if male *C. marginiventris* and *M. rufiventris* are attracted to host-induced plant odors. Naïve females of both species are in fact known to make use of the volatiles produced by plants under herbivore attack to locate their hosts (Turlings *et al.*, 1990; Turlings *et al.*, 2004; Turlings and Wäckers, 2004). Furthermore, *C. marginiventris* female can learn to respond to such odors by association; they show increased attraction to induced plant odors after they perceive them during contact with host feces or during oviposition in a host (Turlings *et al.*, 1989; Turlings *et al.*, 1993; Cormier *et al.*, 1998; Tamò *et al.*, 2006). *M. rufiventris* females show a general increase in responsiveness to plant odor after such an experience, but do not show a preference for the specific odor that they experience (Tamò et al., 2006).

To our knowledge there has been only one study (Baeder and King, 2004) showing associative learning in male parasitoid. They found that male *Nasonia vitripennis*, are attracted to certain colors after they learn to associate them with the presence of virgin females. With this in mind, we decided to test if male *C. marginiventris* and *M. rufiventris* are, similarly to their conspecific females, innately attracted to induced plant odors, and if this attraction can eventually be stimulated and/or enhanced by a contact with conspecific females parasitoids in presence of the concerned plant odors. Our hypothesis was that, if males come in contact with females and perceive the plant odors at the same time, they might associate these odors with the presence of potential mates and subsequently be attracted to them.

Materials and methods

Insects

Cotesia marginiventris and *Microplits rufiventris* were reared on larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae). The rearing colony of *C. marginiventris* originated from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi, USA). The *M. rufiventris* colony was established using individuals collected in 1999 and 2000 from parasitized *S. littoralis* larvae near Alexandria in Egypt. The parasitoids were reared as described in Tamò *et al.* (2006)

Hosts

S. littoralis larvae were reared from eggs provided by Syngenta (Stein, Switzerland). The eggs were kept in an incubator (25 °C and 16L:8D) and after emergence larvae were placed on artificial diet at room temperature until they were used for parasitoid rearing or in the experiments.

Plants

Maize plants (*Zea mays*, var. Delprim) were reared in a climate chamber at 23°C, 60% r. h., 16D: 8L and 50’000 lm/m^2. Two seeds were planted per pot (200ml) in commercial soil (COOP, Switzerland). The day before an experiment, 8-9 day old plants were transplanted in glass pots that fit the olfactometer (250 ml, 4.5 cm diameter, 11 cm high).

Olfactometer experiments

Two experiments were carried out to investigate if the females and the males of the two parasitoid species *C. marginiventris* and *M. rufiventris* were attracted to conspecifics of either sex. With another experiment we investigated if the induced odors emitted by maize plants under caterpillar attack were attractive to naïve and experienced males of both species. For a detailed description of the six-arm olfactomer used see Turlings *et al.*, (2004).

1. Attraction of females and males to conspecifics of either sex

To determine the attraction of adult males and females to adult males and females of the same species we conducted the following experiments using the six-arm olfactometer.

a) In the first experiment, 10 females of either *C. marginiventris* or *M. rufiventris* were placed in one of the arms for 0.5h while plugs of cotton were used to prevent them from either walking up into the trapping bulb or out of the arm into the choice chamber. This way, any odor emitted by the females could accumulate and adsorb onto the glass in the arm. After 0.5h the cotton plugs were removed and the normal experimental airflows were passed through all arms. The wasps were left in the arm and were given another 0.5h to settle in the trapping bulb before starting the actual assays. The five other arms were left empty.

On each test day 3 groups of six females and 3 groups of six males of the correspondent species were released alternately in the choice chamber and their choices were recorded after 30 min. This was repeated for 6 days for each species, each time with new groups of 10 females in a different arm.

b) The second experiment was conducted exactly in the same way, but instead of having the females in one of the arms of the olfactometer, the arm contained10 males. Again on each test day 3 groups of six females and 3 groups of six males were released alternately in the choice chamber and their choices were recorded. This was also repeated on 6 different days, each time with the 10 males in a different arm.

2. Attraction of the males to the induced odors produced by maize plants under caterpillar attack

A third experiment was carried out to investigate if the males of the two parasitoid species were attracted to the odors produced by maize plants under caterpillar attack.
Two maize seedlings fed on by 15 *S. littoralis* larvae during one night served as an odor source in one of the vessels. All other odor source vessels were left empty. On each test day 3 groups of six naïve males and 3 groups of six experienced males of the same species were released alternately in the choice chamber and their choices were recorded after 30 min. The experience was given by placing a male in a plastic container (9.5 cm diam., 5 cm high) with 5 females of the same species and with two maize leaves that had been fed on by twenty 2nd instar *S. littoralis* larvae during one night. The males were kept in the containers during 20 minutes, just before releasing them in the

olfactometer. This experiment was repeated for 6 separate days for each parasitoid species, each time with the odor of caterpillar-damaged maize plants introduced through a different arms. During a bioassay the volatiles emitted by the maize plants were trapped as described in Turlings *et al.* (2004). Before each experiment, the odor collection traps were rinsed five times with 200 µl methylene chloride. Collections always lasted 3.5h. The first olfactometer tests started 0.5h after a collection started. After each collection, the traps were removed, extracted and analyzed as described in Turlings *et al.* (2004).

For all experiments the two parasitoid species were tested on separate days in a semi-random order (also dependent on wasp availability) and after an experimental day, all parts of the olfactometer were water washed and rinsed with acetone and hexane. The glass parts were then placed in a drying oven (250°C) overnight.

Statistical treatment of data

A log linear model assuming quasinormal distribution, thus allowing for overdispersion of the wasps (Turlings *et al.*, 2004), was used to test for differences in the number of choices for the different arms of the olfactometer.

Results

1a. Attractiveness of females (Figure 1)

C. marginiventris

When offered the odor of conspecific females, almost two thirds of the females did not enter an arm and those that did, distributed themselves evenly over all six arms (P = 0.2). In contrast, the majority of the males choose an arm, and the arm containing the females was chosen far more than the empty arms this preference was highly significant (P < 0.001).

M. rufiventris

Approximately half of the females choose one arm within the 30 min., but they did not show a preference for a specific arm (P = 0.953). A great majority (85.2%) of the males did not enter the arms, but those that did, showed a significant preference for the arm containing the females compared to the empty arms (P < 0.01).

1b. Attractiveness of males (Figure 2)

C. marginiventris

Most females entered an arm (71%), but they were not more attracted to the arm containing males than to the other arms (P = 0.437). Only about a third of the males entered an arm and those that did, did not show any preference (P = 0.521).

M. rufiventris

Almost half of the females entered in one of the arms, but no preference was found (P = 0.09). Approximately two thirds of the males did not choose an arm, and the ones that did, did not show a preference (P = 0.272).

2. Attractiveness of the induced maize odors for males (Figure 3)

The analyses confirmed the release of the typical blend of induced maize volatiles (Turlings *et al.*,2004). Despite the fact that the maize plant used for the experiment produced considerable amounts of volatiles (between 2353 ng/3.5h and 8344 ng/3.5h), neither naïve *C. marginiventris* (P = 0.07) nor naïve *M. rufiventris* (P = 0.951) males showed a preferences for the arm carrying the maize odor. This was not changed after the males had had the possibility to associate the presence of females with the odor of host-damaged maize leaves (P = 0.182 and P = 0.834 respectively). Although not significant, there was a tendency for *C. marginiventris* males to avoid the plant odor.

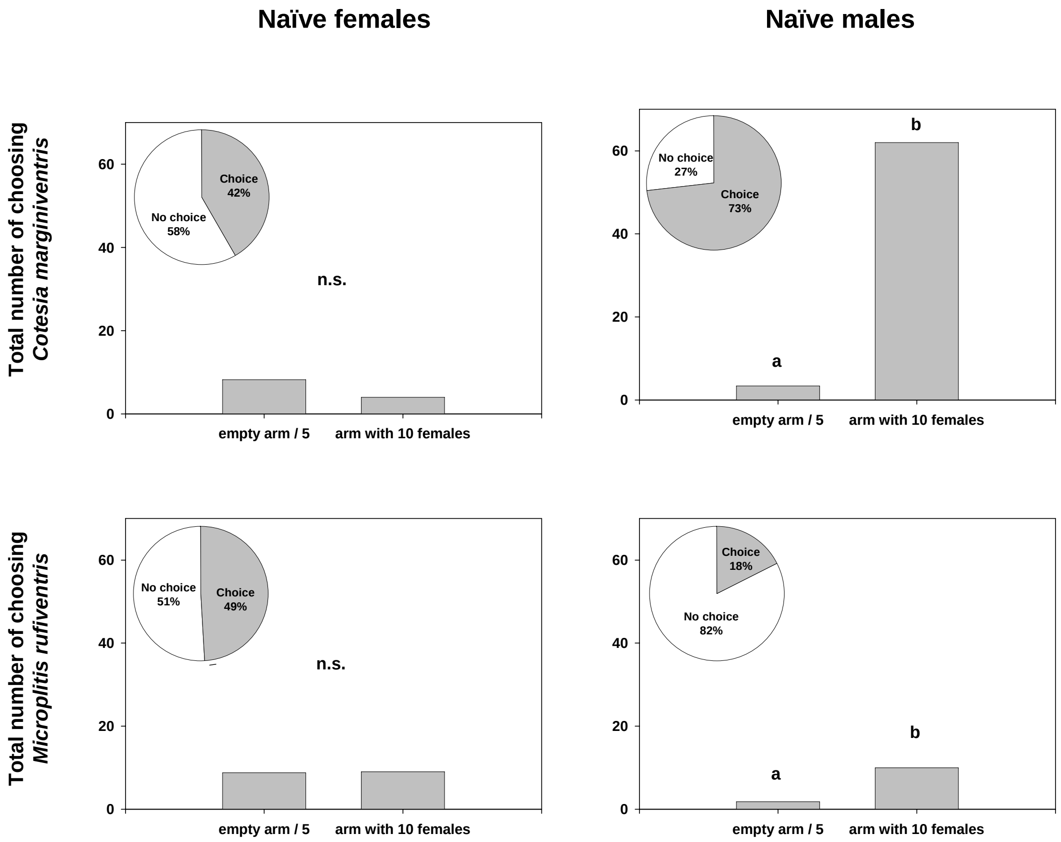


Figure 1. Olfactometric responses of naïve females and males of the parasitoids *Cotesia marginiventris* and *Microplitis rufiventris* to the odours of females conspecific. The pie chart shows the percentage of wasps that entered an arm. The bars represent the total number of parasitoid attracted to the arm containing the conspecific females or of parasitoids that went into one of the empty arms. Choices for the empty arms were summed and divided by 5. Bars having no letters in common represent significantly different response levels within each graph.

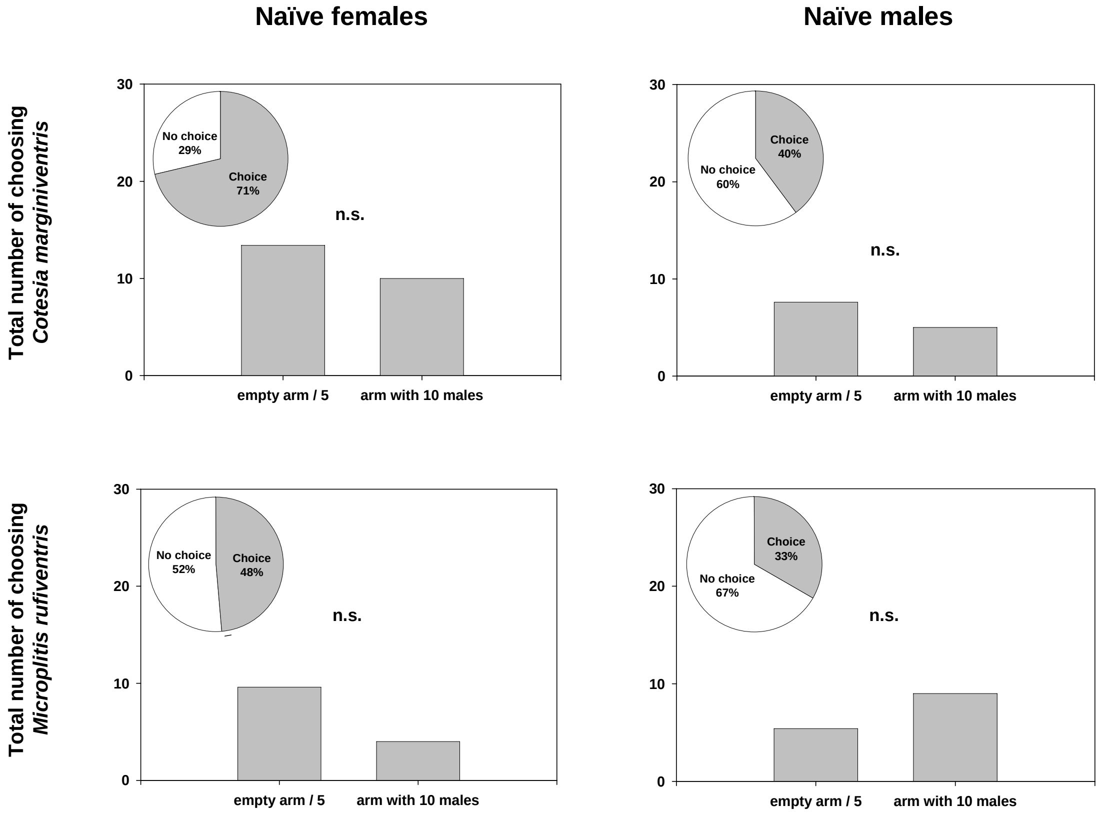


Figure 2. Olfactometric responses of naïve females and males of the parasitoids *Cotesia marginiventris* and *Microplitis rufiventris* to odours of males conspecific. The pie chart shows the percentage of wasps that entered an arm. The bars represent the total number of parasitoid attracted to the arm containing the males conspecific or of parasitoids that went into one of the empty arms. Choices for the empty arms were summed and divided by 5. Bars having no letters in common represent significantly different response levels within each graph.

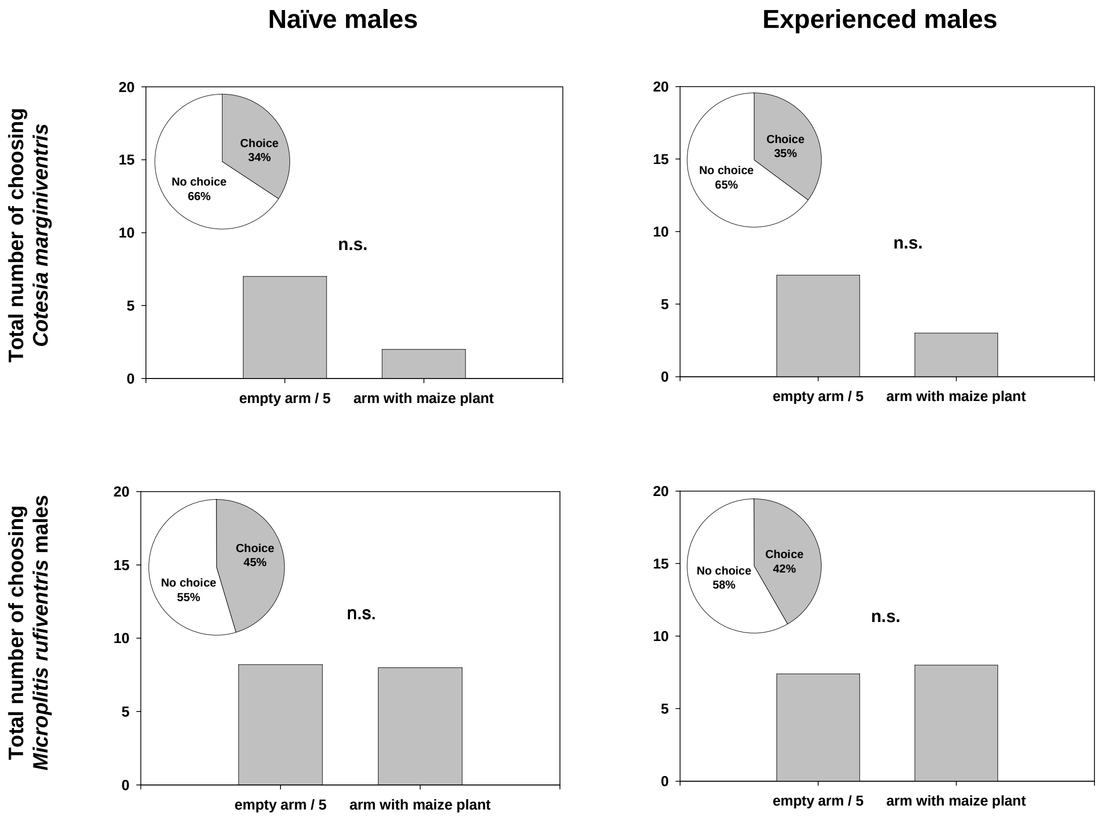


Figure 3. Olfactometric responses of *Cotesia marginiventris* and *Microplitis rufiventris* males to odours of maize infested with *Spodoptera littoralis*. The pie charts show the percentage of wasps that entered an arm. The bars indicate the choices made for the different arms. Choices for the five empty arms were summed and divided by 5. Bars having no letters in common represent significantly different response levels within each graph.

Discussion

C. marginiventris males were found to be strongly attracted to their conspecific females, but females were not attracted to males and females and males were not attractive for conspecific of the same sex. The results for *M. rufiventris* were similar except that the attraction of males to females was much less pronounced. The lack of a strong attraction of *M. rufiventris* males to conspecific females may be attributed to one or more of the following factors: 1) *M. rufiventris* females may produce a sex-pheromone only when they are virgin, as has been found by McNeil and Brodeur (1995) for the aphid parasitoid, *Aphidius nigripes*. However, in other species, like for example *Cotesia flavipes* (De Freitas *et al.*, 2004) and *Ascogaster quadridentata* (DeLury *et al.*, 1999) mated females were found to be just as attractive to conspecific males as virgin females; 2) *M. rufiventris* females could show a temporal pattern of sexual pheromone emission, as it was demonstrated by Cormier *et al.* (1998) for the parasitoid species *Anaphes listronoti*, or 3) females of *M. rufiventris* do not emit a long-range volatile sex pheromone, but attraction occurs only at a short distance, with the use of less volatile compounds, as is the case for the solitary parasitoid *Aphelinus asychis* (Fauvergue *et al.*, 1995). The fact that in the same olfactometer *C. marginiventris* males strongly responded to the odor of females and that *C. marginiventris* and *M. rufiventris* females were found to be very responsive to plant odors (Tamò *et al.*, 2006) indicates that the olfactometer is well-suited for these types studies and that a lack in response is unlikely to be due to methodology.

We cannot yet conclude anything about the nature of the attractants that were involved in the attraction of males to females. It could have been a volatile sex pheromone that was directly emitted by the females, but also in both cases it is possible that the females had deposited less volatile substances during the 0.5 h that they walked in the olfactometer arm before the start of the actual assays. Such substrate-born sex pheromones have been found for *Trichigramma brassicae*, *Ascogaster reticulates* and *Aphelinus asychis* (Kamano *et al.*, 1989; Fauvergue *et al.*, 1995; Pompanon *et al.*, 1997), where the males perceived the chemicals previously emitted and deposited by virgin females on leaves and other substrates. That sex pheromones can strongly adsorb to glass is evident from experiments done by Shu and Jones (1993) with the parasitoid *Eriborus tenebrans* and work on the parasitoid *Trichogramma brassicae* by Pompanon *et al.* (1997).

Substrate borne sex pheromone have been found in several other Braconidae species (Boush and Baerwald, 1967; Askari and Alishah, 1979; De Freitas *et al.*, 2004). Females of the *Cotesia rubecula*, for instance, produce a sex-pheromone in a specialized abdominal gland (Field and Keller, 1994). Because of its close relatedness, something similar may be expected for *C. marginivetris*. The lack of attractiveness for conspecific of the same sex is a phenomenon that has also been already demonstrated in a few species (Syvertsen *et al.*, 1995; DeLury *et al.*, 1999).

Males of both species showed absolutely no attraction to induced maize odors; neither naïve nor experienced males responded to the plant odors. Similar results have been obtained in studies on some unrelated parasitoids. Although the antennae of both males and females of the parasitoids *Roptrocerus xylophygorum*, *R. tutela* and *R. mirus* respond in a very similar way to the odors of trees on which they can find their bark beetle hosts when tested with the electroantennogram recording technique (EAG) (Pettersson *et al.*, 2000; Pettersson, 2001), only females and not males are attracted to these odors. Also the males of the species *Macrocentrus grandii*, contrarily to their conspecific females, are not attracted to corn volatiles or volatiles of other host plant (Udayagiri and Jones, 1992; Jones, 1996). Finally, males of the parasitoid *Diachasmimorpha longicaudata* are not attracted to the odors of the fruit that attracts females (Messing and Jang, 1992). It appears that, unlike several herbivorous insects, these parasitoids do not use plant odors as cues to locate mates, not even after experiencing the odors during a successful encounter with females. For male wasps, visual cues from plants may be more important than odor cues. For instance, McAuslane *et al.* (1990) found that for the males of *Campoletis sonorensis*, both the olfactory and the visual cues played a role in oriented flights of the males toward cotton plants in a wind tunnel. However, in their experiments, almost twice as many males located a female on a visible plant, compared to the number of males that located a female in the presence of only the volatiles cues produced by the plant.

Recently Baeder and King (2004) found the first evidence for associative learning by male parasitoid wasps. Using males of the parasitoid species *Nasonia vitripennis,* they showed that males develop a preference for a color after having learned to associate it with a reward of virgin females. In the six-arm olfactometer the plants are not visible to the parasitoids it remains therefore possible that plant-provided visual cues play a role in mate finding, possibly in synergy with odors. Concluding, we can say that for both *C. marginiventris* and *M. rufiventris* males plant odors alone do not to play a role in mate finding.

Acknowledgements

We thank the Swiss National Science Foundation (grant 31-058865.99) and the Swiss National Centre of Competence in Research “Plant Survival” for supporting our work. We are grateful to all members of the laboratoire d’entomologie évolutive (University of Neuchâtel) for their continuous support and scientific feedback. We specially thank Yves Borcard and Liselore Roelfstra for the parasitoids rearing, Syngenta (Basel, Switzerland) for the weekly shipment of *S. littoralis*, Donald Nordlund for providing *C. marginiventris*, Esmat Hegazi for providing *M. rufiventris* and Matthias Held for aid with the statistics.

References

Askari, A. and Alishah, A. (1979). Courtship behavior and evidence for a sex-pheromone in *Diaeretiella rapae* (Hymenoptera, Braconidae), the Cabbage Aphid primary parasitoid. *Ann. Entomol. Soc. Am.* **72**: 749-50.

Baeder, J.M. and King, B.H. (2004). Associative learning of color by males of the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *J. Insect Behav.* **17**: 201-13.

Boush, G.M. and Baerwald, R.J. (1967).. *Ann. Entomol. Soc. Am.* **60:** 865-866.

Cormier, D., Royer, L., Vigneault, C., Panneton, B. and Boivin, G. (1998). Effect of female age on daily cycle of sexual pheromone emission in gregarious egg parasitoid *Anaphes listronoti*. *J. Chem. Ecol.* **24**: 1595-1610.

De Freitas, M.D.T., Mendonca, A.D., do Nascimento, R.R. and Sant'ana, A.E.G. (2004). Behavioural evidence for a female sex pheromone in *Cotesia flavipes* (Hymenoptera: Braconidae). *Physiol. Entomol.* **29**: 183-187.

DeLury, N.C., Gries, G., Gries, R., Judd, G.J.R. and Brown, J.J. (1999). Sex pheromone of *Ascogaster quadridentata*, a parasitoid of *Cydia pomonella*. *J. Chem. Ecol.* **25**: 2229-2245.

Eller, F.J., Bartelt, R.J., Jones, R.L. and Kulman, H.M. (1984). Ethyl (Z)-9-hexadecenoate a sex-pheromone of *Syndipnus rubiginosus*, Hymenoptera, Ichneumonidae a sawfly parasitoid. *J. Chem. Ecol.* **10**: 291-300.

Eller, F.J., Tumlinson, J.H. and Lewis, W.J. (1988). Beneficial arthropod behavior mediated by airborne semiochemicals.2. Olfactometric studies of host location by the parasitoid *Microplitis croceipes* (Cresson) (Hymenoptera, Braconidae). *J. Chem. Ecol.* **14**: 425-434.

Fauvergue, X., Fleury, F., Lemaitre, C. and Allemand, R. (1999). Parasitoid mating structures when hosts are patchily distributed: field and laboratory experiments with *Leptopilina boulardi* and *L. heterotoma*. *Oikos* **86**: 344-356.

Fauvergue, X., Hopper, K.R. and Antolin, M.F. (1995). Mate finding via a trail sex-pheromone by a parasitoid wasp. *Proc. Nat. Acad. Sci. USA* **92**: 900-904.

Field, S.A. and Keller, M.A. (1993) Courtship and intersexual signaling in the parasitic wasp *Cotesia rubecula* (Hymenoptera, Braconidae). *J. Ins. Beh.* **6**: 737-750.

Field, S.A. and Keller, M.A. (1994). Localization of the females sex pheromone gland in *Cotesia rubecula* Marshall (Hymenoptera: Braconidae). *J. Hymenopt. Res.* **3**: 151-156.

Jones, R.L. (1996). Semiochemicals in host and mate finding behavior of *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae). *Florida Entomol.* **79**: 104-108.

Kainoh, Y., Nemoto, T., Shimizu, K., Tatsuki, S., Kusano, T. and Kuwahara, Y. (1991) Mating-behavior of *Ascogaster reticulatus* Watanabe (Hymenoptera, Braconidae), an egg-larval parasitoid of the Smaller Tea Tortrix, *Adoxophyes* Sp (Lepidoptera, Tortricidae).3. Identification of a sex-pheromone. *Appl. Entomol. Zool.* **26**: 543-549.

Kamano, Y., Shimizu, K., Kainoh, Y. and Tatsuki, S. (1989). Mating-behavior of *Ascogaster reticulatus* Watanabe (Hymenoptera, Braconidae), an egg-larval parasitoid of the Smaller Tea Tortrix, *Adoxophyes* Sp (Lepidoptera, Tortricidae). 2. Behavioral sequence and a role of sex-pheromone. *Appl. Entomol. Zool.* **24**: 372-378.

Kawaguchi, M. and Tanaka, T. (1999). Biological characteristics of a larval endoparasitoid, *Cotesia plutellae* (Hymenoptera: Braconidae): Host stage preference, subsequent sex ratio of progeny and mate location of males. *Appl. Entomol. Zool.* **34**: 213-221.

Kimani, S.W. and Overholt, W.A. (1995). Biosystematics of the *Cotesia flavipes* Complex (Hymenoptera, Braconidae) - Interspecific hybridization, sex-pheromone and mating-behavior studies. *Bull. Entomol. Res.* **85**: 379-386.

Landolt, P.J., Heath, R.R., Millar, J.G., Davishernandez, K.M., Dueben, B.D. and Ward, K.E. (1994). Effects of host-plant, *Gossypium hirsutum* L, on sexual attraction of Cabbage Looper Moths, *Trichoplusia ni* (Hubner) (Lepidoptera, Noctuidae). *J. Chem. Ecol.* **20**: 2959-2974.

Landolt, P.J. and Phillips, T.W. (1997). Host plant influences on sex pheromone behavior of phytophagous insects. *Ann. Rev. Entomol.* **42**: 371-391.

Lecomte, C. and Pouzat, J. (1985). EAG responses of 2 Ichneumonid parasitoids, *Diadromus pulchellus* and *Diadromus collaris*, to odors emitted by plants, the phytophagous-host *Acrolepiopsis assectella* and the sexual partner. *Entomol. Exp. Appl.* **39**: 295-306.

Lewis, W.J., Snow, J.W. and Jones, R.L. (1971). Pheromone trap for studying populations of *Cardiochiles nigriceps*, Hymenoptera-Braconidae a parasite of *Heliothis virescens* Lepidoptera Noctuidae. *J.Econ. Entomol.* **64**: 1417-1421.

Li, Y.S., Dickens, J.C. and Steiner, W.W.M. (1992). Antennal olfactory responsiveness of *Microplitis croceipes* (Hymenoptera, Braconidae) to cotton plant volatiles. *J. Chem. Ecol.* **18**: 1761-1773.

McAuslane, H.J., Vinson, S.B. and Williams, H.J. (1990). Influence of host plant on mate location by the parasitoid *Campoletis sonorensis* (Hymenoptera, Ichneumonidae). *Environ. Entomolo.* **19**: 26-31.

McNeil, J.N. and Brodeur, J. (1995). Pheromone-mediated mating in the aphid parasitoid, *Aphidius nigripes* (Hymenoptera, Aphididae). *J. Chem. Ecol.* **21**: 959-972.

Messing, R.H. and Jang, E.B. (1992). Response of the fruit-fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera, Braconidae) to host-fruit stimuli. *Environ. Entomol.* **21**: 1189-1195.

Mohamed, M.A. and Coppel, H.C. (1987). Pheromonal basis of courtship behavior in 2 Gypsy-Moth parasitoids - *Brachymeria intermedia* (Nees) and *Brachymeria lasus* (Walker) (Hymenoptera, Chalcididae). *J. Chem. Ecol.* **13**: 1099-1113.

Ochieng, S.A., Park, K.C., Zhu, J.W. and Baker, T.C. (2000). Functional morphology of antennal chemoreceptors of the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). *Arthropod Struc. Dev.* **29**: 231-240.

Park, K.C., Zhu, J.W., Harris, J., Ochieng, S.A. and Baker, T.C. (2001). Electroantennogram responses of a parasitic wasp, *Microplitis croceipes*, to host-related volatile and anthropogenic compounds. *Physiol. Entomol.* **26**: 69-77.

Pettersson, E.M. (2001). Volatile attractants for three Pteromalid parasitoids attacking concealed spruce bark beetles. *Chemoecol.* **11**: 89-95.

Pettersson, E.M., Sullivan, B.T., Anderson, P., Berisford, C.W. and Birgersson, G. (2000). Odor perception in the bark beetle parasitoid *Roptrocerus xylophagorum* exposed to host associated volatiles. *J.Chem. Ecol.* **26**: 2507-2525.

Pompanon, F., DeSchepper, B., Mourer, Y., Fouillet, P. and Bouletreau, M. (1997). Evidence for a substrate-borne sex pheromone in the parasitoid wasp *Trichogramma brassicae*. *J. Chem. Ecol.* **23**: 1349-1360.

Ramachandran, R. and Norris, D.M. (1991). Volatiles mediating plant-herbivore-natural enemy interactions - Electroantennogram responses of Soybean Looper, *Pseudoplusia includens,* and a parasitoid, *Microplitis demolitor*, to green leaf volatiles. *J. Chem. Ecol.* **17**: 1665-1690.

Reed, H.C., Tan, S., Reed, D.K., Elliott, N.C., Burd, J.D. and Walker, T. (1994). Evidence for a sex attractant in *Aphidus colemani* Viereck with potential use in field studies. *Southwestern Entomol.* **19**: 273-278.

Reinecke, A., Ruther, J. and Hilker, M. (2005). Electrophysiological and behavioural responses of *Melolontha melolontha* to saturated and unsaturated aliphatic alcohols. *Entomol. Exp. Appl.* **115**: 33-40.

Robacker, D.C. and Hendry, L.B. (1977). Neral and geranial - Components of sex-pheromone of parasitic wasp, *Itoplectis conquisitor*. *J. Chem. Ecol.* **3**: 563-577.

Rungrojwanich, K. and Walter, G.H. (2000). The Australian fruit fly parasitoid *Diachasmimorpha kraussii* (Fullaway): Mating behavior, modes of sexual communication and crossing tests with *D. longicaudata* (Ashmead) (Hymenoptera: Braconidae: Opiinae). *Pan-Pacific Entomol.* **76**: 12-23.

Salom, S.M., Birgersson, G., Payne, T.L. and Berisford, C.W. (1991). Electroantennogram responses of the southern Pine-Beetle parasitoid *Dinotiscus dendroctoni* (Ashmead) (Hymenoptera, Pteromalidae) to potential semiochemicals. *J. Chem. Ecol.* **17**: 2527-2538.

Shu, S.Q. and Jones, R.L. (1993). Evidence for a multicomponent sex-pheromone in *Eriborus terebrans* (Gravenhorst) (Hym, Ichneumonidae), a larval parasitoid of the European Corn-Borer. *J. Chem. Ecol.* **19**: 2563-2576.

Sullivan, B.T. (2002) Evidence for a sex pheromone in bark beetle parasitoid *Roptrocerus xylophagorum*. *J. Chem. Ecol.* **28**: 1045-1063.

Swedenborg, P.D. and Jones, R.L. (1992). Multicomponent sex-pheromone in *Macrocentrus grandii* Goidanich (Hymenoptera, Braconidae). *J. Chem. Ecol.* **18**: 1901-1912.

Swedenborg, P.D., Jones, R.L., Zhou, H.Q., Shin, I.J. and Liu, H.W. (1994). Biological activity of (3r,5s,6r)3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one and

(3s,5r,6s)3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one, a pheromone of *Macrocentrus grandii* (Goidanich) (Hymenoptera, Braconidae). *J. Chem. Ecol.* **20**: 3373-3380.

Syvertsen, T.C., Jackson, L.L., Blomquist, G.J. and Vinson, S.B. (1995). Alkadienes mediating courtship in the parasitoid *Cardiochiles nigriceps* (Hymenoptera: Braconidae). *J. Chem. Ecol.* **21**: 1971-1989.

Tamò, C. (2006). *A comparative study on the plant odour preferences and learning ability of three solitary endoparasitoids of Spodoptera species*, Ph.D. Thesis, University of Neuchâtel.

Turlings, T.C.J. and Benrey, B. (1998). Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience* **5**: 321-333.

Turlings, T.C.J., Davison, A.C. and Tamò, C. (2004). A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiol. Entomol.* **29**: 45-55.

Turlings, T.C.J., Tumlinson, J.H. and Lewis, W.J. (1990). Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250**: 1251-1253.

Turlings, T.C.J., Tumlinson, J.H., Lewis, W.J. and Vet, L.E.M. (1989). Beneficial arthropod behavior mediated by airborne semiochemicals. 8. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *J. Insect Behav.* **2**: 217-225.

Turlings, T.C.J., Waeckers, F., Vet, L.E.M., Lewis, W.J. and Tumlinson, J.H. (1993). Learning of host-finding cues by hymenopterous parasitoids. In Papaj, D.R. and Lewis, A. (eds.), *Insect Learning: Ecological and Evolutionary Perspectives*, Chapman and Hall, New York, pp. 51-78.

Turlings, T.C.J. and Wäckers, F.L. (2004). Recruitment of predators and parasitoids by herbivore-damaged plants. In Cardé, R. T., and Millar, J. (eds.), *Advances in Insect Chemical Ecology*, Cambridge University Press, Cambridge, pp. 21-75.

Udayagiri, S. and Jones, R.L. (1992). Flight behavior of *Macrocentrus grandii* Goidanich (Hymenoptera, Braconidae), a specialist parasitoid of European Corn-Borer (Lepidoptera, Pyralidae) - factors influencing response to corn volatiles. *Environ. Entomol.* **21**: 1448-1456.

van den Assem, J. (1986). Mating behaviour in parasitic wasps. In Waage, J., and Greathead, D. (eds.), *Insect Parasitoids*, Academic Press, London, pp. 137-67.

Vet, L.E.M. and Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* **37:** 141-172.

Vet, L.E.M., Lewis, W.J. and Cardé, R.T. (1995). Parasitoid foraging and learning. In Cardé, R. T., and Bell, W. J. (eds.), *Chemical Ecology of Insects 2*, Chapman and Hall, New York, pp. 65-101.

Vinson, S.B. (1972). Courtship behavior and evidence for a se pheromone in the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Environ. Entomol.* **1**: 409-414.

Whitman, D.W. (1988). Allelochemical interactions among plants, herbivores, and their predators. In Barbosa, P., and Letourneau, D. (eds.), *Novel Aspects of Insect-Plant Interactions*, John Wiley and Sons, New York, pp. 207-48.

A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours

Based on: Cristina Tamò, Ingrid Ricard, Matthias Held, A. C. Davison and Ted C. J. Turlings. A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours, *Animal Biology* (2006) 56, 205-220.

Animal Biology, Vol. 56, No. 2, pp. 205-220 (2006)

A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours

CRISTINA TAMÒ [1], INGRID RICARD [2], MATTHIAS HELD [1],
ANTHONY C. DAVISON [2], TED C.J. TURLINGS [1,*]

[1] *Université de Neuchâtel, Institut de Zoologie, Entomologie Évolutive, Case Postale 158, 2009 Neuchâtel, Switzerland*
[2] *Swiss Federal Institute of Technology, Institute of Mathematics, 1015 Lausanne, Switzerland*

Abstract—Many parasitic wasps that exploit herbivores as their hosts make use of herbivore-induced plant odours to locate their victims and these wasps often exhibit an ability to learn to associate specific plant-produced odours with the presence of hosts. This associative learning is expected to allow generalist parasitoids to focus on cues that are most reliably associated with current host presence, but evidence supporting this hypothesis is ambiguous. Using a six-arm olfactometer we compared the responses of three generalist larval endoparasitoids, *Cotesia marginiventris* (Hymenoptera: Braconidae), *Microplitis rufiventris* (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Hymenoptera: Ichneumonidae), to the induced odours of three plant species: maize (*Zea mays*), cowpea (*Vigna unguiculata*), and cotton (*Gossypium hirsutum*). We tested the responses of naïve females as well as of females that were first conditioned by parasitising host larvae feeding on one of the plant species. Despite similarities in biology and host range the three wasp species responded entirely differently. Naïve *C. marginiventris* and *C. sonorensis* chose equally among the induced odours of the three plants, whereas naïve *M. rufiventris*, which may have a somewhat more restricted host range, tended to prefer the odour of maize. After conditioning, most *C. marginiventris* females chose the odour of the plant species that they had experienced, but conditioned *M. rufiventris* showed an even stronger preference for maize odours, independently of the plant they had experienced. *Cotesia sonorensis* did not show any change in its preference after conditioning. We speculate that its extremely broad host range allows *C. sonorensis* females to use fixed responses to cues commonly associated with plants damaged by Lepidoptera. These results imply that different generalist parasitoids may employ different foraging strategies and that associative learning is not necessarily part of it.

Keywords: associative learning; herbivore-induced plant odours; host finding; parasitoids.

---

[*] Corresponding author; e-mail: ted.turlings@unine.ch

INTRODUCTION

Many parasitoids of herbivores use plant odours induced by insect feeding to locate plants that may carry their hosts (Vet and Dicke, 1992; Turlings and Benrey, 1998). Associative learning of the most reliable volatile cues may help the wasps to find such plants more efficiently (Turlings et al., 1993; Vet et al., 1995). Parasitoids can learn to associate a specific odour with a successful oviposition in a host or contact with host faeces. This ability to learn is generally expected to be an adaptive strategy for parasitoids that have a broad host range or which can find their hosts on multiple plant species (Vet and Groenewold, 1990; Vet and Dicke, 1992; Vet, 1999; Steidle and van Loon, 2003). Indeed, generalist parasitoids are often found to exhibit this learning ability, whereas there is a tendency for parasitoids with a more restricted host range (or a limited range of plants on which they find hosts) to show genetically fixed responses to host location cues (Steidle and van Loon, 2003). The general validity of the concept, however, is still unclear (Steidle and van Loon, 2003). More insight may be obtained by comparing the effect of experience in multiple generalist parasitoid species under similar conditions. This was the aim of the current study, which compares the responses of naïve and conditioned females of the three generalist parasitoids *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae), *Microplitis rufiventris* Kok. (Hymenoptera: Braconidae) and *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) to host-induced plant odours.

Cotesia marginiventris and *C. sonorensis* have been extensively studied for their abilities to make use of plant odours to locate their hosts and for their ability to learn such odours. Dmoch et al. (1985) showed that a contact experience with host and/or host by-products enhances the responses of *C. marginiventris* females to frass, silk and feeding damage of one of its hosts, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), on maize leaves. For long-range host finding, *C. marginiventris* relies primarily on volatiles emitted by plants after an attack by caterpillars (Turlings et al., 1990; Turlings et al., 1991). The intensity with which they respond to the plant-provided signals depends on previous experiences and they learn to prefer the odours they encounter in the presence of hosts (Turlings et al., 1990; Hoballah and Turlings, 2005).

Campoletis sonorensis is a solitary parasitoid that co-occurs with *C. marginiventris* and shows a great overlap in the range of host species it attacks (Hoballah et al., 2004). Studies on the flight behaviour of *C. sonorensis* have shown that it orients to microhabitat cues without requiring a prior experience with host or microhabitat stimuli (Elzen et al., 1986; Baehrecke et al., 1990). McAuslane et al. (1991) found that *C. sonorensis* females that were given an oviposition experience in host larvae on a particular plant were subsequently more responsive in flight tunnel assays than naïve females. However, such experiences with a host-plant complex did not change the wasps' innate preference for a particular plant odour, suggesting that *C. sonorensis* does not learn to associate specific odours with host presence (McAuslane et al., 1991).

Studies on *M. rufiventris* have mostly focused on its physiology and development (Altahtawy et al., 1976b; Hegazi et al., 1977; Tawfik et al., 1980; Hegazi and Shabaan, 1984). From previous studies we know that naïve females of *M. rufiventris* are also highly attracted to induced maize odours (Hoballah and Turlings, 2005; Tamò, 2006), but the effect of experience on this behaviour is unclear (Hoballah and Turlings, 2005). The behaviour of some other *Microplitis* species has been studied more extensively and indicates a keen ability of each species to learn to respond to host cues by association (Drost et al., 1986; Hérard et al., 1988; Murray and Rynne, 1994). It is unlikely, however, that the recorded behaviour of a particular species can be extrapolated to other species belonging to the same genus. Different *Cotesia* species, for example, show clear differences in their responses to host cues (Potting et al., 1997; Geervliet et al., 1998). Moreover, different *Microplitis* species have distinctly different host ranges and are therefore likely to have evolved different host searching strategies.

Cotesia marginiventris and *C. sonorensis* are native to the Americas and *M. rufiventris* is mainly found in the Middle East, but these three parasitoid species have much in common. They are generalist solitary larval endoparasitoids that attack the early stages of a wide range of Noctuidae species on many different plants. *Campoletis sonorensis* is an ichneumonid parasitoid with at least 27 species of noctuid larvae recorded as hosts (Lingren et al., 1970). *Cotesia marginiventris*, a braconid, also attacks a wide range of noctuid species, many in common with *C. sonorensis* (Turlings, 1990). The host range of *M. rufiventris*, also a braconid, is less well known and may be more restricted. It has been reported to oviposit and successfully develop in young instars of various *Spodoptera* species and *Heliothis armigera* on many different crop plants (El-Minshawy, 1963; Hammad et al., 1965; Gerling, 1969; Abou Zeid et al., 1978).

Many of the hosts of the three parasitoids belong to the genus *Spodoptera*. *Cotesia marginiventris* and *C. sonorensis* can attack *S. frugiperda*, *S. exigua* and *S. eridania* (Lingren et al., 1970; Turlings, 1990), while *M. rufiventris* attacks *S. littoralis*, *S. litura*, *S. exempta*, *S. mauritia*, *S. latebrosa* and *S. exigua* (Meier, 1929; Thompson, 1946; El-Minshawy, 1963; Hammad et al., 1965; Gerling, 1969; Altahtawy et al., 1976a). Most *Spodoptera* species are polyphagous and attack many plant species including important crops like alfalfa, cotton, cowpea, maize, tomato and soybean (Brown and Dewhurst, 1975; Hodge et al., 1983). Hence, the three parasitoids should be able to locate hosts on a multitude of plant species and can be expected to show similarities in how they forage for hosts. A direct comparison between these species may provide insight into how similar or how variable host searching strategies are among generalist parasitoids.

With this objective, we tested responses of *C. marginiventris*, *C. sonorensis* and *M. rufiventris* to the odours of three different host plants that are readily attacked by *Spodoptera* larvae, and that can be encountered by the parasitoids in their native habitats. The plant species of choice were maize (*Zea mays*, var. Delprim), cotton (*Gossypium herbaceum*) and cowpea (*Vigna unguiculata*). The caterpillar-induced odours produced by these plants have already been analysed in various studies (Turlings et al., 1991; McCall et al., 1994; Loughrin et al., 1995; Röse et al., 1996; Paré and Tumlinson, 1997; Hoballah et al., 2002) and show distinct differences in quality (composition) and quantity. The preference of naïve females for the induced volatiles of the three plants was compared with the preference of females that had an oviposition experience on one of the plant-host-complexes. During the behavioural assays part of the emitted odours were collected and subsequent analyses made it possible to correlate the observed behavioural responses to the identities and quantities of the volatiles that the wasps perceived in the olfactometer. Females of all three species were found to be strongly attracted to the induced plant odours and as naïve wasps they showed no clear preferences, but their responses after experience were distinctly different.

METHODS

Plants

Three host plants were used for the experiments: maize (*Zea mays*, var. Delprim), cotton (*Gossypium herbaceum*) and cowpea (*Vigna unguiculata*). For each plant species two seeds were planted per pot (200 ml) with fertilised commercial soil (COOP, Switzerland) and placed in a climate chamber (E15 Conviron, Winnipeg, Canada) set at 23°C, 16 D : 8 L, 60% relative humidity and 50 000 lm/m^2 light intensity. Plants were watered daily. The day before an experiment, two plants of each species were transplanted in glass pots that fit the olfactometer (250 ml, 4.5 cm diam., 11 cm high). Maize and cowpea plants were 8-10 days old and cotton 13-14 days old at day of testing. Plants were induced to emit volatiles by placing 15 2nd instar *S. littoralis* larvae on them on the evening before an experimental day.

Host larvae

Spodoptera littoralis eggs were supplied by the Syngenta rearing facilities (Stein, Switzerland). After emergence the larvae were reared on a wheatgerm-based artificial diet (also supplied by Syngenta) at room temperature. *Spodoptera littoralis* larvae at the L2 stage, the preferred stage for female parasitoids of all three species (Altahtawy et al., 1976b; Isenhour, 1985; Jalali et al., 1987), were used in experiments.

Parasitoids

The rearing strain of *C. marginiventris* came from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi, USA) and was refreshed with individuals collected in Mexico in 2001. The *M. rufiventris* colony was established in 2000 from individuals collected from parasitised *S. littoralis* larvae in fields in Egypt (Alexandria). The *C. sonorensis* colony was established in 2001 from individuals collected from parasitised *S. frugiperda* larvae in maize fields in Mexico (Poza Rica, Veracruz). All parasitoids were reared on *Spodoptera littoralis* (Noctuidae: Lepidoptera). For the rearing, 25 *S. littoralis* caterpillars were offered to two mated females (4-7 days old) for 3-4 h in a plastic container (9.5 cm diam., 5 cm high). The parasitised caterpillars were then maintained in groups of 100 individuals in transparent plastic boxes (15 × 13 × 5.5 cm) on the wheatgerm-based artificial diet (supplied by Syngenta) until cocoon formation. Cocoons were kept in Petri dishes until adult emergence. Upon emergence, adults were sexed and kept in cages (30 × 30 × 30 cm) (MegaView Science Education Services Co. Ltd., Taiwan) at a sex ratio of 1:2 (male: female). They were supplied with honey droplets as food source and moist cotton. Parasitised larvae and adults of *C. marginiventris* were held until the experimental day in an incubator (E36L Percival, Perry, Iowa, USA) at 25°C and 16 L : 8 D, whereas parasitised larvae and adults of *M. rufiventris* and *C. sonorensis* were held in the laboratory under ambient light and temperature conditions (19-24°C).

Olfactometer set-up

The responses of females of the three parasitoids species *C. marginiventris*, *M. rufiventris* and *C. sonorensis* to volatile chemicals emitted by the three plant species maize, cotton and cowpea were investigated in the six-arm olfactometer described in Turlings et al. (2004). Three arms of the olfactometer had always only clean air and they were alternated with arms with the odour of either two maize seedlings, two cotton seedlings or two cowpea seedlings fed upon by 15 2nd instar *S. littoralis* larvae. For each replicate the odour sources were connected to a different arm, but always with one empty chamber between two chambers that contained two plants of one species. After an experimental day, all parts of the olfactometer were water washed and rinsed with acetone and hexane. The glass parts were then placed in a drying oven (250°C) overnight.

Bioassay procedure

Parasitoid species were tested on separate days in a roughly random order, depending on availability of the wasps. On a given day, four groups of six wasps of one species were released one after the other in random order and choices made within each group were recorded 30 min after release. The four groups of wasps were the following: i) naïve females (no previous contact with hosts or plants); ii) females experienced with maize; iii) females experienced with cotton; and iv) females experienced with cowpea. The experience was given by letting a female parasitise two larvae in a plastic box (9.5 cm diam., 5 cm high) containing a leaf of one of the three plant species, which had been fed upon by 15 2nd instar *S. littoralis* larvae during one night. Within 20 min after six females had had such an experience, they were released in the olfactometer. After their choices had been recorded, the six wasps were removed just prior to the release of the next group. The sequence of the different groups of wasps released in the olfactometer was randomly changed for each replicate day ($n = 12$). It should be noted that in previous experiments we have tested for the possibility that individual females affect each other's choice, as described for *C. marginiventris* by Turlings et al. (2004). In none of the cases did we find such an effect but, interestingly, there is an interspecies interference (C. Tamò, 2006). Even though we feel that we can rule out overdispersion due to interference between the wasps, our statistical analysis corrects for this possibility (see below).

Statistical analyses of the olfactometer data were based on a log-linear model, but in comparison with the method described by Turlings et al. (2004) the statistical analysis was improved by using a stochastic model developed specifically to allow for the significant overdispersion of the olfactometer data relative to that seen in a standard log-linear model. The new model takes into account the censored aspect of the data: some of the wasps did not make a choice within the given time for a release. The number of wasps choosing the ith arm ($i = 1, \ldots, 6$) indicates the relative attractiveness of the corresponding odour source, which is parameterised by λ_i. If only the odour source affects wasp choice, and this effect is the same for all wasp species and experiences, the corresponding model is:

$$\log \lambda_i = \beta_{\mathrm{p}} \tag{1}$$

where β_{p} measures the attractiveness of either an empty arm or of the odour source in the ith arm.

As the results for *C. marginiventris* suggested a preference for an experienced odour, we adapted the model to assume that a wasp, which has already encountered a plant may subsequently be more attracted to this plant (fig. 1A):

$$\log \lambda_{\mathrm{e,p}} = \beta_{\mathrm{p}} + \gamma \mathrm{I} \quad (\mathrm{e} = \mathrm{s} \neq \mathrm{n}) \tag{2}$$

where $\mathrm{I}\,(\mathrm{e} = \mathrm{s} \cdot \mathrm{n}) = 1$ if the odour previously experienced is the same as the odour in the corresponding arm and $\mathrm{I}\,(\mathrm{e} = \mathrm{s} \cdot \mathrm{n}) = 0$ otherwise; here e = experience, s = odour source, and n = naïve.

The parameter γ describes the magnitude of this effect and will differ from zero when previous experience of an odour influences the wasps' attraction to this odour. A significant difference between the fits of models (1) and (2) would indicate that the wasps are indeed more attracted to an odour they previously experienced.

In order to assess whether experienced wasps will, independent from the odour they experienced, always prefer maize, as seemed to be the case for *M. rufiventris* (fig. 1B), we used the model:

$$\log \lambda_{\mathrm{e,p}} = \beta_{\mathrm{p}} + \gamma \mathrm{I} \quad (\mathrm{e} \neq \mathrm{n}, \mathrm{s} = \mathrm{m}) \tag{3}$$

with m = maize odour.

A significant difference between models (1) and (3) would suggest that experienced wasps always have a higher preference for maize than naive wasps. In all

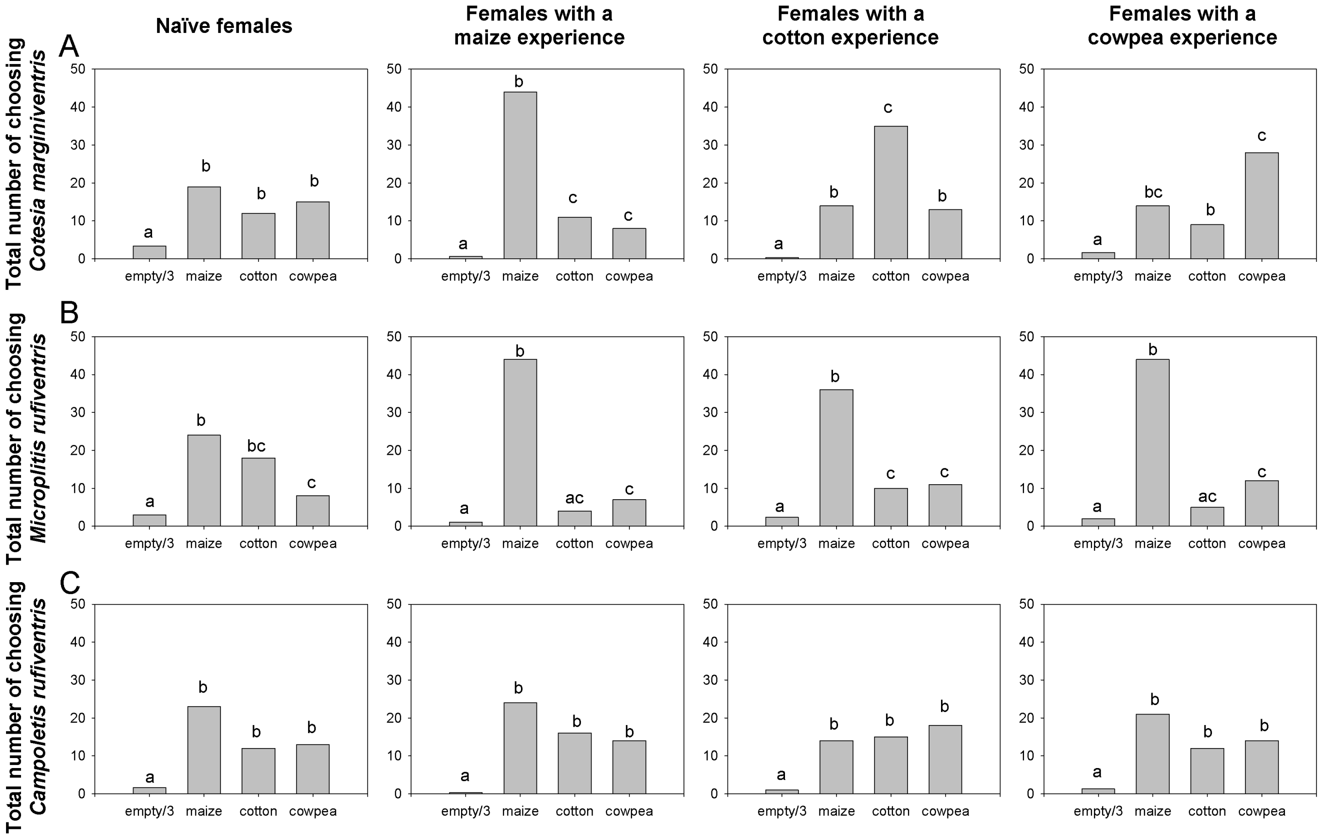


Figure 1. Olfactometric responses of the parasitoids *Cotesia marginiventris* (A), *Microplitis rufiventris* (B) and *Campoletis sonorensis* (C) when given a choice between the odours of *Spodoptera littoralis*-infested maize, cotton and cowpea plants. The wasps were either naïve or had had two oviposition experiences in hosts that were feeding on one of the three plant species. Bars having no letters in common represent significantly different response levels within each graph.

cases, we estimated the parameters by maximising the log-likelihood and compared the different models through likelihood ratio tests.

Volatile collections

During all experiments 50% of the air passing over the odour sources was pulled for 3 h through a trapping filter containing 25 mg of 80-100 mesh Super Q adsorbent (Alltech, Deerfield, Illinois) as described by Turlings et al. (1998, 2004). Immediately after each experiment, the volatiles collected on the filters were extracted with 150 μl of methylene chloride and two internal standards (n-octane and nonyl acetate, each 200 ng in 10 μl methylene chloride) were added to these extracts. The samples were either analysed immediately or stored at −70°C for later analysis. They were analysed by gas chromatography and mass spectrometry as described by Turlings et al. (2004). The purpose of these collections and analyses was to confirm the presence of the typical caterpillar-induced odours for each of the three plant species during each of the experiments.

HP GC Chemstation software was used to quantify all major components by comparison to the known quantity of internal standards. A Kruskal-Wallis ANOVA on ranks and Tukey post-hoc test were used to compare the total quantities of collected volatiles among the plant species.

RESULTS

Wasp behaviour

All naïve and experienced parasitoid females showed a strong preference for the arms containing the odour of infested plants as compared to the control arms (fig. 1). Naïve *C. marginiventris* females had no obvious preference for a particular plant odour and chose equally among the three species ($P = 0.1$). After experience, the majority of *C. marginiventris* females showed a significant preference for the odour that they had experienced ($P < 0.001$; fig. 1A). In contrast, naïve *M. rufiventris* females tended to prefer the odour of *S. littoralis*-damaged maize plants and this preference for maize was greatly enhanced after experience, independently of the plant species used to train the females ($P < 0.001$; fig. 1B). *C. sonorensis* females, whether naïve or experienced, did not show a preference for a particular plant odour produced by one of the three plant species attacked by *S. littoralis* larvae ($P = 0.7$; fig. 1C).

A statistical comparison between the three species may be compromised by the fact that they were tested on different days and to different individual plants. However, as the order of the experiments was more or less random (depending on wasp availability) and there were many replications (12 per experience type, each with six wasps), a day or plant effect seems very unlikely. Moreover, the volatile collections revealed that there was no difference in what the plants emitted when the experimental days were compared for the three wasps species (see below). Using the log-linear model for comparison between the three species showed a highly significant difference ($P < 0.001$) in the effect of experience on responses among the three wasps.

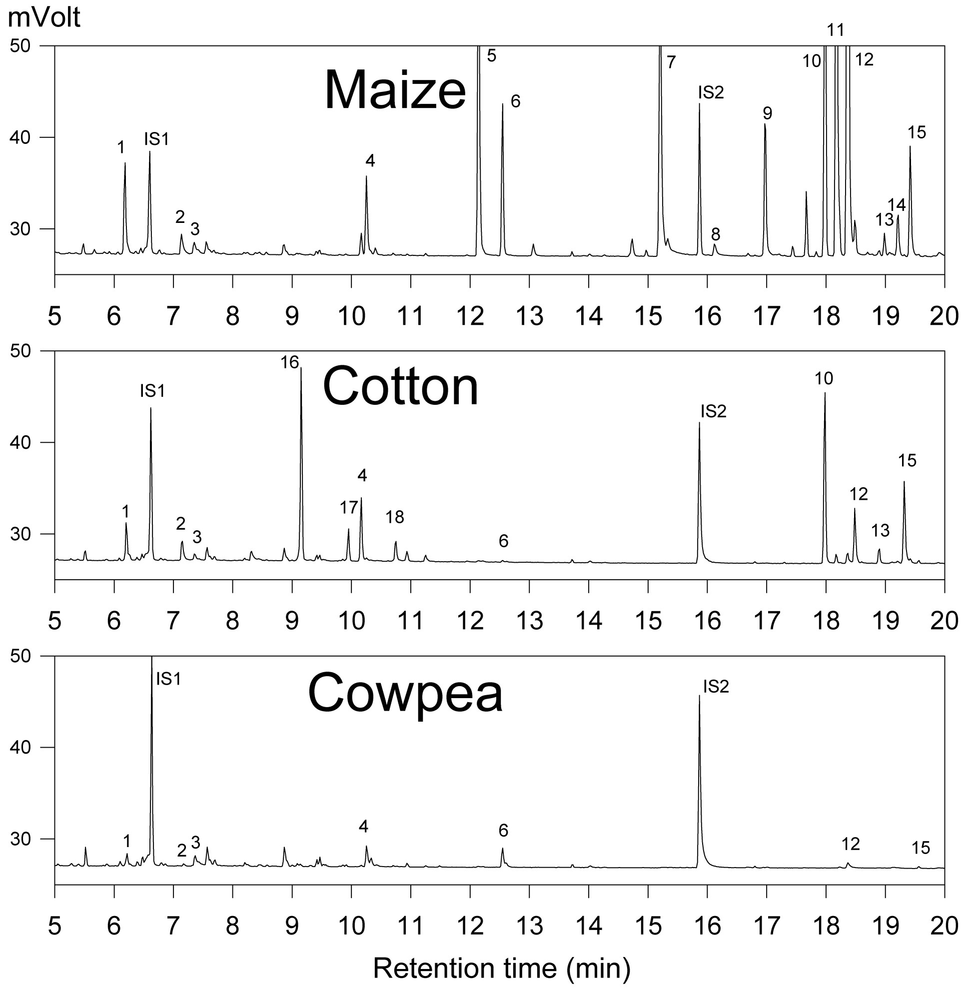
Odour emissions

The analyses of the odours collected during the bioassays produced volatile profiles (fig. 2A) that were very similar to those collected in previous studies (Turlings and Wäckers, 2004). The blends emitted by the three plants differed both in composition and quantity, all of them releasing typical green leafy volatile, but different blends of terpenoids and a few aromatic compounds (fig. 2A). The total quantity of produced volatiles was approximately four times higher for maize than for cotton, and about five times higher for cotton than for cowpea (fig. 2B) and these differences were highly significant (Two-way ANOVA, $F_{2,99} = 93.96$; $P < 0.001$). Comparing between the collections obtained from the experiments with the three wasps species revealed no differences (Two-way ANOVA, $F_{2,99} = 0.257$; $P = 0.774$), implying that they were exposed to similar odours. The quantitative differences between the three plant species were not reflected in the wasps' responses (compare figs 1, 2).

DISCUSSION

The ability of parasitoids to learn by association is well documented (Vet and Dicke, 1992; Turlings et al., 1993; Vet et al., 1995; Steidle and van Loon, 2003) and, according to Steidle and van Loon (2003), is expected to occur mostly in parasitoids with a broad host range. Our comparison of three taxonomically different, but ecologically similar parasitoids shows that such a generality cannot be readily made. Despite similarities in their biology and host range, *C. marginiventris*, *M. rufiventris*, and *C. sonorensis* showed clear differences in how they exploit volatiles that may guide them to their hosts. A recent study on the antennal perception shows that all three perceive most of the volatiles that the plants emit (Guouinguené et al., 2005), but obviously they use this information differently. The three wasps had somewhat similar innate responses, but their odour preferences were entirely different after experiencing a particular odour in association with successful ovipositions.

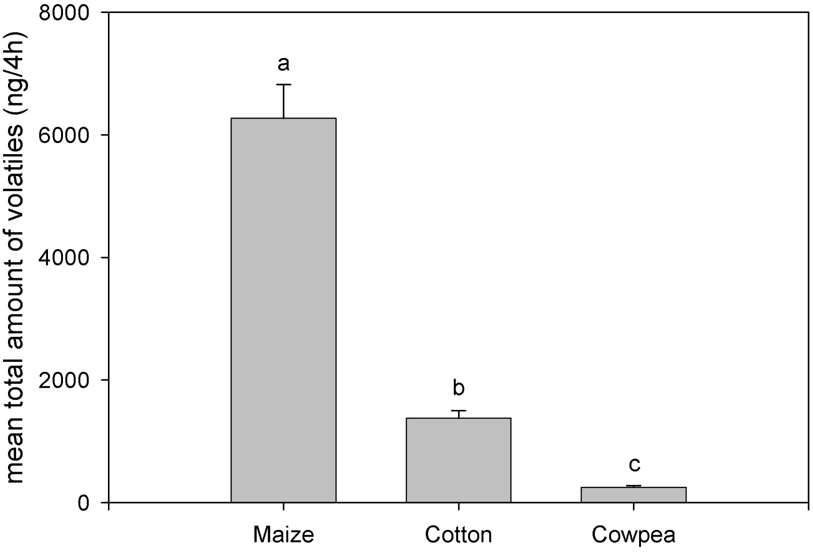
Naïve *C. marginiventris* and *C. sonorensis* chose equally among the induced odours of the three plants, whereas naïve *M. rufiventris* showed a slight preference for maize and cotton odours. After experience, *C. marginiventris* always preferred the odour of the plant species that they had experienced, very much in line with the "varying response model" proposed by Vet et al. (1990). In contrast, experienced *M. rufiventris* increased its preference for maize odour, independently of the plant they had experienced, and *C. sonorensis* showed no change in its preference after experience.



(A)

Figure 2. **A**. Typical chromatographic profiles of the volatiles of the induced plants that were collected during the bio-assays. The labelled peaks are: **1**, (*Z*)-3-hexenal; **2**, (*E*)-2-hexenal; **3**, (*Z*)-3-hexenol; **4**, (*Z*)-3-hexenyl acetate; **5**, linalool; **6**, (*E*)-4,8-dimethyl-1,3,7-nonatriene; **7**, indole; **8**, methyl anthranilate; **9**, geranyl acetate; **10**, (*E*)-β-caryophyllene; **11**, (*E*)-α-bergamotene; **12**, (*E*)-β-farnesene; **13**, (E,E)-α-farnesene; **14**, nerolidol; **15**, (*E,E*)-4,8,12-trimethyl-1,3,7, 11-tridecatetraene; **16**, α-pinene; **17**, β-pinene; **18**, (*E*)-β-ocimene. Two internal standards, n-octane and nonyl-acetate, are labelled with **IS1** and **IS2**, respectively. **B**. The average total amounts of volatiles collected for the three plant species for all experiments. The letters indicate significant differences between the plants.

Thus, *C. marginiventris* showed a clear ability of associative learning, increasing its responsiveness to the odour of the plant-host complex it experienced, as has been observed in previous studies (Turlings et al., 1989, 1990, 1993). Additional studies now show that this parasitoid innately responds to plant volatiles that are associated



(B)

Figure 2. (Continued).

with fresh leaf damage (Hoballah and Turlings, 2005) and not to the truly induced volatiles such as the terpenoids that the plants release only several hours after initial caterpillar attack (D'Alessandro and Turlings, 2005a). The response to the typically induced volatiles apparently only occurs after *C. marginiventris* females have associated such volatiles with the presence of hosts (Hoballah and Turlings, 2005). This should allow a newly emerged wasp to first explore damaged plants of various species for host presence and after successful host location focus its search efforts on the most profitable plant species.

Apparently, *M. rufiventris* and *C. sonorensis* follow different strategies. Experienced *M. rufiventris* females appear to use cues that are closely associated with the host rather than the general plant volatile blend. This is evident from studies whereby naïve females were observed to be strongly attracted to artificially induced maize odours, but after an oviposition experience in the presence of a natural herbivore-induced maize odour blend, their response to the artificially induced blend was much lower and they strongly prefer the natural odour of plants under attack by caterpillars (Hoballah and Turlings, 2005; Tamò, 2005). If indeed they learn to respond to cues that come directly from the host or its by-products (which are missing from the emissions of artificially induced plants) it could be that these host-related cues are best perceived from hosts on maize plants. It should be noted that of the three species tested, *M. rufiventris* appears to have the most restricted host range. To our knowledge only seven host species have been reported for *M. rufiventris* (El-Minshawy, 1963; Hammad et al., 1965; Gerling, 1969; Abou Zeid et al., 1978), but this species has been relatively poorly studied and a broader host range is likely. Nevertheless, it could be considered more of a specialist than the other two, which could explain a more specific use of host location cues (Vet and Dicke, 1992; Steidle and van Loon, 2003). However, more specialized species of the same genus have been shown to be very good learners (Drost et al., 1986; Hérard et al., 1988; Lewis et al., 1991; Murray and Rynne, 1994).

C. sonorensis, on the other hand, is, like *C. marginiventris*, an extreme generalist (Lingren et al., 1970), and the fact that its responses were not affected by experience is therefore in clear contrast to the expected use of learning by generalists. It remains possible that this wasp changes it preferences after more or different types of experiences, or that the absence of an effect is a particularity of the specific strain we used. However, the lack of change in the preference of *C. sonorensis* after experience is consistent with an earlier study by McAuslane et al. (1991), who used an entirely different strain. We cannot rhyme this behaviour with the expected behavioural flexibility in generalists. One could speculate that *C. sonorensis* uses very common plant cues that are nevertheless reliably associated with caterpillar presence. Further studies will have to be conducted with manipulated odour blends to pinpoint the volatile cues this species actually uses.

The analysis of the volatiles collected during the bioassays show that the induced volatiles produced by the three plant species differed both in quantity and in composition. The total amount of produced volatiles was approximately four times higher for maize than for cotton, and about five times higher for cotton than for cowpea (fig. 2B), and the composition of the blends clearly differed among plant species (fig. 2A). As already found by Hoballah et al. (2002), both quantity and composition of the odours affects the response of *C. marginiventris* females. If only the quantity had played a role, than the majority of the females of each species should have chosen the arms containing maize plants. *M. rufiventris* indeed showed a preference for maize volatiles, However, the above mentioned previous experiments (Hoballah and Turlings, 2005; Tamò, 2005) indicate a more complicated effect of experience, whereby it appears that the females increase their responsiveness to cues that are directly related to the host. If this is indeed the case, the observed preference for maize could be due to more of these cues being produced by the host on this plant. The preference for maize in *M. rufiventris* cannot be explained by the origin of the strain used for the experiments, as our lab colony originated from cotton fields in Egypt and host presence and natural parasitisation rates are high on cotton (Hegazi et al., 1973; Ibrahim and Tawfik, 1975).

The considerable differences in responses to host-induced plant odours observed here imply that we cannot readily generalise about how parasitoids have evolved to optimise host microhabitat location. The studied wasps have comparable host ranges and life cycles, but showed considerable differences in how experiences affected their responses to the odours of host-infested plants. This confirms the notion of Steidle and van Loon (2003) that the expectation that generalist parasitoids make use of associatively learning, and that specialists exhibit genetically fixed responses, does not always hold true. Our knowledge on parasitoid host foraging strategies is also largely hampered by the fact that little is known about which compounds are the key attractants in the complex plant-produced blends. Novel behavioral, chemical and molecular techniques may help to resolve this gap in our knowledge (Degenhardt et al., 2003; D'Alessandro and Turlings, 2006b).

ACKNOWLEDGEMENTS

We thank the members of the *Laboratoire d'Entomologie Évolutive* (University of Neuchâtel) for their continuous support and scientific feedback. We greatly benefited from the comments by three reviewers of an earlier version of the manuscript. We wish to further acknowledge the support of our work by the Swiss National Science Foundation (grant 31-058865.99) and the Swiss National Centre of Competence in Research 'Plant Survival'.

REFERENCES

Abou Zeid, N.A., El-Dakroury, M.S.I., El-Heneidy, A.H. & Abbas, M.S.T. (1978) Biology of *Microplitis rufiventris* Kok. parasitizing *Heliothis armigera* Hb. in Egypt. *Agric. Res. Rev. (Cairo)*, 56, 31-36.

Altahtawy, M.M., Hammad, S.M. & Hegazi, E.M. (1976a) Studies on the dependence of *Microplitis rufiventris* Kok (Hym, Braconidae) parasitizing *Spodoptera littoralis* (Boisd) on own food as well as on food of its host. *Z. Angew. Entomol.-J. Appl. Entomol.*, 81, 3-13.

Altahtawy, M.M., Hegazi, E.M. & Hammad, S.M. (1976b) Convenience of ages and instars of *Spodoptera littoralis* (Boisd) larvae for *Microplitis rufiventris* Kok – Mass rearing and biological-control of Cotton Leafworm. *Z. Angew. Entomol.-J. Appl. Entomol.*, 81, 270-285.

Baehrecke, E.H., Vinson, S.B. & Williams, H.J. (1990) Foraging behavior of *Campoletis sonorensis* in response to *Heliothis virescens* and cotton plants. *Entomol. Exp. Appl.*, 55, 47-57.

Brown, E.S. & Dewhurst, C.F. (1975) The genus *Spodoptera* (Lepidoptera, Noctuidae), in Africa and the Near East. *Bull. Entomol. Res.*, 65, 221-262.

D'Alessandro, M. & Turlings, T.C.J. (2005) *In situ* modification of herbivore-induced plant odours: a novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. *Chem. Senses*, 30, 1-15.

D'Alessandro, M. & Turlings, T.C.J. (2006) Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *The Analyst*, 131, 24-32.

Degenhardt, J., Gershenzon, J., Baldwin, I.T. & Kessler, A. (2003) Attracting friends to feast on foes: engineering terpene emission to make crop plants more attractive to herbivore enemies. *Curr. Opin. Biotechnol.*, 14, 169-176.

Dmoch, J., Lewis, W.J., Martin, P.B. & Nordlund, D.A. (1985). Role of host-produced stimuli and learning in host selection behavior of *Cotesia* (= *Apanteles*) *marginiventris* (Cresson) (Hymenoptera, Braconidae). *J. Chem. Ecol.*, 11, 453-463.

Drost, Y.C., Lewis, W.J., Zanen, P.O. & Keller, M.A. (1986) Beneficial arthropod behavior mediated by airborne semiochemicals. 1. Flight behavior and influence of preflight handling of Microplitis croceipes (Cresson). *J. Chem. Ecol.*, 12, 1247-1262.

El-Minshawy, A.M. (1963) *Studies on the morphology and biology of* Spodoptera exigua *Hb. and* Spodoptera latebrosa. Faculty of Agriculture, Dissertation, University of Alexandria.

Eller, F.J., Tumlinson, J.H. & Lewis, W.J. (1992) Effect of host diet and preflight experience on the flight responses of *Microplitis croceipes* (Cresson). *Physiol. Entomol.*, 17, 235-240.

Elzen, G.W., Williams, H.J. & Vinson, S.B. (1986) Wind-tunnel flight responses by Hymenopterous parasitoid *Campoletis sonorensis* to cotton cultivars and lines. *Entomol. Exp. Appl.*, 42, 285-289.

Geervliet, J.B.F., Vreugdenhil, A.I., Dicke, M. & Vet, L.E.M. (1998). Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula*. *Entomol. Exp. Appl.*, 86, 241-252.

Gerling, D. (1969) The parasites of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) eggs and larvae in Israel. *Isr. J. Entomol.*, 4, 73-81.

Hammad, S.M., El-Minshawy, A.M. & Salama, A. (1965) Studies on *Microplitis rufiventris* Kok. (Hym., Braconidea). *Bull Soc. Entomol. Egypte*, 49, 215-219.

Gouinguené, S., Pickett, J.A., Wadhams, L.J., Birkett, M.A. & Turlings, T.C.J. (2005) Antennal electrophysiological responses of three parasitic wasps to the caterpillar-induced volatiles from maize, cowpea and cotton. *J. Chem. Ecol.*, 31, 1023-1038.

Hegazi, E.M., Elminshawy, A.M. & Hammad, S.M. (1977) Suitability of *Spodoptera littoralis* larvae for development of *Microplitis rufiventris*. *J. Agric. Sci.*, 89, 659-662.

Hegazi, E.M., Hammad, S.M., Altahtawy, M.M. & El-Sawaf, S.K. (1973) Parasites of the larval stage of the cotton leaf-worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) in Alexandria region. *Z. Angew. Entomol.-J. Appl. Entomol.*, 74, 332-336.

Hegazi, E.M. & Shabaan, M.A. (1984) Competition and the superparasitism of *Spodoptera littoralis* (Boisd.) larvae by *Microplitis rufiventris* Kok. *J. Agric. Sci.*, 103, 181-187.

Hérard, F., Keller, M.A., Lewis, W.J. & Tumlinson, J.H. (1988) Beneficial arthropod behavior mediated by airborne semiochemicals. 4. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson (Hymenoptera, Braconidae). *J. Chem. Ecol.*, 14, 1597-1606.

Hoballah, M.E., Degen, T., Bergvinson, D., Savidan, A., Tamò, C. & Turlings, T.C.J. (2004) Occurrence and direct control potential of parasitoids and predators of the fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico. *Agric. For. Entomol.*, 6, 83-88.

Hoballah, M.E. & Turlings, T.C.J. (2005) The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. *J. Chem. Ecol.*, 31, 2003-2018.

Hoballah, M.E.F., Tamo, C. & Turlings, T.C.J. (2002) Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: Is quality or quantity important? *J. Chem. Ecol.*, 28, 951-968.

Hodges, R.W., Dominick, T., Davis, D.R., Ferguson, D.C., Franclemont, J.G., Munroe, E.G. & Powell, J.A. (1983) *Checklist of the Lepidoptera of America And North of Mexico*. Washington, D.C., The Wedge Entomological Research Foundation, 284 pp.

Ibrahim, M. & Tawfik, K. (1975) *A report on the survey of natural enemies attacking the American bollworm.* Cairo, Agricultural Research Center, Plant Protection Research Institute.

Isenhour, D.J. (1985) *Campoletis sonorensis* Hym, Ichneumonidae as a parasitoid of *Spodoptera frugiperda* Lep, Noctuidae – Host stage preference and functional response. *Entomophaga*, 30, 31-36.

Jalali, S.K., Singh, S.P. & Ballal, C.R. (1987) Studies on host age preference and biology of exotic parasite, *Cotesia marginiventris* (Cresson) (Hymenoptera, Braconidae). *Entomon*, 12, 59-62.

Lewis, W.J., Tumlinson, J.H. & Krasnoff, S. (1991) Chemically mediated associative learning – an important function in the foraging behavior of *Microplitis croceipes* (Cresson). *J. Chem. Ecol.*, 17, 1309-1325.

Lingren, P.D., Guerra, R.J., Nickelse, J.W. & White, C. (1970) Hosts and host-age preference of *Campoletis perdistinctus* (Hymenoptera, Ichneumonidae). *J. Econ. Entomol.*, 63, 518-522.

Loughrin, J.H., Manukian, A., Heath, R.R. & Tumlinson, J.H. (1995) Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *J. Chem. Ecol.*, 21, 1217-1227.

McAuslane, H.J., Vinson, S.B. & Williams, H.J. (1991) Influence of adult experience on host microhabitat location by the generalist parasitoid, *Campoletis sonorensis* (Hymenoptera, Ichneumonidae). *J. Insect Behav.*, 4, 101-113.

McCall, P.J., Turlings, T.C.J., Loughrin, J., Proveaux, A.T. & Tumlinson, J.H. (1994) Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L) seedlings. *J. Chem. Ecol.*, 20, 3039-3050.

Meier, N.F. (1929) Schlupfwespen die in Russland in den Jahren 1881-1926 aus Schaedlingen gezogen sind (Fortsetzung). *Izv. Otd. Prokl. Entomol.*, 4, 231-248.

Murray, D.A.H. & Rynne, K.P. (1994) Effect of host-plant on parasitism of *Helicoverpa armigera* (Lep, Noctuidae) by *Microplitis demolitor* (Hymenoptera, Braconidae). *Entomophaga* 39, 251-255.

Paré, P.W. & Tumlinson, J.H. (1997) De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiol.*, 114, 1161-1167.

Potting, R.P.J., Otten, H. & Vet, L.E.M. (1997) Absence of odour learning in the stemborer parasitoid *Cotesia flavipes*. *Anim. Behav.*, 53, 1211-1223.

Röse, U.S.R., Manukian, A., Heath, R.R. & Tumlinson, J.H. (1996) Volatile semiochemicals released from undamaged cotton leaves – A systemic response of living plants to caterpillar damage. *Plant Physiol.*, 111, 487-495.

Steidle, J.L.M. & van Loon, J.J.A. (2003) Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomol. Exp. Appl.*, 108, 133-148.

Tamò, C. (2006) *A comparative study on the plant odour preferences and learning ability of three solitary endoparasitoids of* Spodoptera *species.* Dissertation, University of Neuchâtel.

Tawfik, M.F.S., Hafez, M. & Ibrahim, A.A. (1980) Immature stages of *Microplitis rufiventris* Kok (Hym, Braconidae). *Dtsch. Entomol. Z.*, 27, 39-50.

Thompson, W.R. (1946) *A catalogue of the parasites and predators of insect pests. Section 1: Host-parasite catalogue. Part 7: Parasites of the Lepidoptera.* Commonwealth Institute of Biological Control, Ontario, Canada.

Turlings, T.C.J. (1990) *Semiochemically mediated, host-searching behavior of the endoparasitic wasp* Cotesia marginiventris *(Cresson) (Hymenoptera: Braconidae).* Dissertation, University of Florida.

Turlings, T.C.J. & Benrey, B. (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience*, 5, 321-333.

Turlings, T.C.J. & Wäckers, F.L. (2004) Recruitment of predators and parasitoids by herbivore-damaged plants. In: R.T. Cardé & J. Millar (Eds.), *Advances in Insect Chemical Ecology*, pp. 21-75. Cambridge, Cambridge University Press.

Turlings, T.C.J., Davison, A.C. & Tamò, C. (2004) A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiol. Entomol.*, 29, 45-55.

Turlings, T.C.J., Lengwiler, U.B., Bernasconi, M.L. & Wechsler, D. (1998) Timing of induced volatile emissions in maize seedlings. *Planta*, 207, 146-152.

Turlings, T.C.J., Tumlinson, J.H., Eller, F.J. & Lewis, W.J. (1991) Larval-damaged plants – Source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. *Entomol. Exp. Appl.*, 58, 75-82.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, 250, 1251-1253.

Turlings, T.C.J., Tumlinson, J.H., Lewis, W.J. & Vet, L.E.M. (1989) Beneficial arthropod behavior mediated by airborne semiochemicals. 8. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *J. Insect Behav.*, 2, 217-225.

Turlings, T.C.J., Wäckers, F., Vet, L.M., Lewis, W.J. & Tumlinson, J.H. (1993) Learning of host-finding cues by hymenopterous parasitoids. In: D.R. Papaj & A. Lewis (Eds.), *Insect Learning: Ecological and Evolutionary Perspectives*, pp. 51-78. New York, Chapman & Hall.

Vet, L.E.M. (1999) From chemical to population ecology: Infochemical use in an evolutionary context. *J. Chem. Ecol.*, 25, 31-49.

Vet, L.E.M. & Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.*, 37, 141-172.

Vet, L.E.M. & Groenewold, A.W. (1990) Semiochemicals and learning in parasitoids. *J. Chem. Ecol.*, 16, 3119-3135.

Vet, L.E.M., Lewis, W.J. & Cardé, R.T. (1995) Parasitoid foraging and learning. In: R.T. Cardé & W.J. Bell (Eds.), *Chemical Ecology of Insects 2*, pp. 65-101. New York, Chapman & Hall.

Vet, L.E.M., Lewis, W.J., Papaj, D.R. & van Lenteren (1990) A variable-response model for parasitoid foraging behavior. *J. Insect Behav.*, 3, 471-490.

Zanen, P.O. & Cardé, R.T. (1991) Learning and the role of host-specific volatiles during in- flight host-finding in the specialist parasitoid *Microplitis croceipes*. *Physiol. Entomol.*, 16, 381-389.

Chapter 4

Effect of host diet and oviposition experience on host searching and host acceptance behavior of the parasitoid *Cotesia marginiventris*

Based on: Cristina Tamò, Lise-Lore Roelfstra, Guillaume Suzanne and Ted C. J. Turlings. Effect of host diet and oviposition experience on host searching respectively and host acceptance behavior of the parasitoid *Cotesia marginiventris*. In preparation for submission to *Journal of Insect Behavior*.

Abstract

In an earlier study we documented that in choice experiments naïve females of the generalist parasitoid *Cotesia marginiventris* are equally attracted to the host-induced odors emitted by the three plant species maize, cotton and cowpea. However, after an oviposition experience with host larvae feeding on one of these plants, female wasps showed a preference for the experienced odors. In the present study we considered two other aspects that could affect the foraging decision of *C. marginiventris* females: 1) the effect of pre-imaginal experience on the responses of naïve adult females to plant induced odors and 2) the effect of an oviposition experience in the presence of a specific plant-host-complex on the subsequent host acceptance behavior of adult females. To test the first, *C. marginiventris* were reared on *Spodoptera* larvae feeding either on an artificial diet, or on leaves from either maize, cotton or cowpea. The responses of naïve females coming from these four rearing groups were then compared in a six-arm olfactometer in which the induced odors of the three plant species were offered simultaneously. No significant effect of pre-imaginal experience was found and none of the treatment groups showed a preference for an odor.

The effect of ovipositional experience on one of four different substrates (three host plants and artificial diet) on the host acceptance was examined in no-choice tests. Independently of the type of experience, all females started to parasitize faster and parasitized more when the offered larvae were feeding on one of the host plants than when larvae were feeding on artificial diet. Only females that had an oviposition experience on artificial diet parasitized the larvae feeding on artificial diet as readily as the others. No differences were found among plant species, implying that, at short range, plant stimuli enhance oviposition drive, but that this is the result of priming (increasing "motivation") rather than associative learning.

Introduction

Various natural enemies of herbivores make use of plant-produced volatiles to locate their prey or hosts (Turlings and Benrey, 1998; Dicke and Vet, 1999; Turlings and Wäckers, 2004). In parasitoids, the attraction to these volatiles can be affected by oviposition experiences that result in odor learning by adult females parasitoids and plays an important role in microhabitat (host-plant) location (Vet, 1987; Vet and Groenewold, 1990; Turlings et al., 1993; Vet et al., 1995) and in host acceptance (Vet and Schoonman, 1988). Several studies have shown that also the preimaginal experiences during the development inside the host can affect the wasps' behavior as adults (Monteith, 1958; Vet, 1983; Drost et al., 1986; Drost et al., 1988; Hérard et al., 1988; Wickremasinghe and van Emden, 1992; Cortesero and Monge, 1994; Bogahawatte and van Emden, 1996; Storeck et al., 2000).

To test the impact of preimaginal experience on the adult behavior of parasitoid females, some studies compared the behavior of adult females that developed as larvae in hosts feeding either on artificial diet or on a host plant. In certain cases, for example for the generalist parasitoid *Campoletis sonorensis* (McAuslane et al., 1990a), no effect on the host microhabitat location was found in the adult female wasps. In other studies female parasitoids responded poorly to volatiles from host/plant complexes if they had been reared on an artificial diet, whereas females reared on a host plant responded much stronger to the odors of that plant. This is the case for *Leptolina clavipes* (Vet, 1983), *Microplitis croceipes* (Drost et al., 1986; Drost et al., 1988), and *M. demolitor* (Hérard et al., 1988). These examples suggests that on unnatural diets immature parasitoids of these species may lack experience with vital information necessary for effective performance as adults.

Other studies have looked at the response of adult parasitoids reared from hosts fed with different plant species. Again, in some cases the dietary history (food plant species) had no effect on the preference of the adult parasitoids, like for *Cotesia glomerata* (Benrey et al., 1997; Geervliet et al., 1998) and *Campoletis sonorensis* (McAuslane et al., 1990a), whereas other species showed a preference for the host-plant complex on which they had been reared, like the parasitoids *Aphidius colemani* (Storeck et al., 2000), *Aphidius rophalosiphi* (Wickremasinghe and van Emden, 1992), *Cotesia plutellae* (Bogahawatte and van Emden, 1996), *Drino bohemica* (Monteith, 1958) and *Eupelmus vuilleti* (Cortesero and Monge, 1994). Hence, again the importance of exposure to host or host plant cues prior to adult emergence seem to be different for different parasitoid species.

An effect of the host's food plant on the behavior of an adult wasp can be due to either 1) larval conditioning, where chemical cues are incorporated and/or may cause neural changes in the parasitoid larva that persist to the adult stage (Corbet, 1985) or 2) early-adult conditioning, where at emergence from the pupa and/or cocoon, the wasp perceives and learns essential cues (Hérard et al., 1988). Few studies have tried to separate these two aspects. Hérard et al. (1988) found that the higher responsiveness to the odors of a plant-host complex of *Microplitis demolitor* adults reared on plant material compared with the responsiveness of adults reared on artificial diet was due to contact with stimuli adsorbed onto the cocoon at the time of the emergence. Studying the species *Eupelmus vuilleti*, Cortesero and Monge (1994) found that the different responses of adult females reared in different Leguminosae seeds was due to early adult learning, which occurs while the imago is in the larval chamber of its host, a bruchid, just before emergence. Similarly, van Emden and colleagues found for several aphid parasitoids that their normal preference for the odor of a plant on which they were reared was no longer there if the pupae were excised from the aphid mummies just before emergence of the females (van Emden et al., 1996; Storeck et al., 2000). They conclude that the normal preference must be determined by the chemicals contacted by a parasitoid on the skin of the mummy or while biting its way out of the dead aphid and they termed this "emergence conditioning". A study with *Hyssopus pallidus* appears to demonstrate true preimaginal learning (Gandolfi et al., 2003). This ectoparasitoid of the coddling moth was observed to learn only during the very early preimaginal developmental stages and not during any of the subsequent stage.

The last decision step, after host habitat location and host finding, in the host selection process by parasitoids is host acceptance (Vinson, 1998). During this phase, females parasitoids use various volatile and non-volatile cues coming directly from their hosts or their by-products (kairomones), but their behavior can also be affected by host-plant cues (synomones) (Vet, 1987; Vet and Schoonman, 1988; Bjorksten and Hoffmann, 1995; Song et al., 1997; Duan and Messing, 1999). In this context Vet and Schoonman (1988) showed that for the parasitoid *Leptopilina heterotoma* associative learning after an oviposition experience in hosts on a particular substrate not only increases the response to the odors produced by this substrate, but also increases the acceptance of the hosts subsequently encountered on the same food substrate. Similarly, Mattiacci et al. (2000) found that for the parasitoid *Cydia pomonella* the odor emitted by the host-food plant not only represents a habitat location signal, but also enhance the host acceptance and oviposition rate, thus directly effecting its reproductive success.

C. marginiventris is a generalist solitary larval endoparasitoid. Plant volatiles induced by caterpillar feeding are the primary cues used by *C. marginiventris* females to locate plants with potential hosts (Turlings et al., 1990). Once on a plant with hosts, the females use contact cues in host faeces and silk to determine the exact location of hosts (Loke and Ashley, 1984a, 1984b). The responses to these contact cues is genetically fixed and the cues in faeces are probably used as unconditioned stimuli in an associative learning process during which the surrounding odor (plant volatiles) is learnt for subsequent host habitat location (Turlings et al., 1989). Because of its wide host range and the numerous potential volatile signals it may use, it is expected that *C. marginiventris* is flexible in its responses. In previous studies it was indeed demonstrated that *C. marginiventris* females show a clear ability of associative learning, increasing their responsiveness to the induced odors of the plant-host complex they experienced by contact with a host or host faeces, or by oviposition of the host larvae (Turlings et al., 1989; Turlings et al., 1990; Turlings et al., 1993; Tamò, 2006). It is as yet unclear, however, if pre-imaginal experience can also affect the attraction of adult *C. marginiventris* females to different host plant blends and if an experience with a host-plant complex as adult can affect their host acceptance behavior.

In the current study, using a six arm olfactometer described by Turlings et al. (2004), we first investigated if the diet (food plant) of the host in which a *C. marginiventris* female develops has an effect on its odor preferences as an adult. Secondly, in no choice experiments it was tested if host acceptance and oviposition rate by *C. marginiventris* on different substrates is affected by previous oviposition experiences on one of these substrates.

Materials and Methods

Growing of plants

Three plant species were used for the experiments: maize (*Zea mays*, var. Delprim), cotton (*Gossypium herbaceum*) and cowpea (*Vigna unguiculata*). Two seeds of each plant species were grown in plastic pots (200ml) with fertilised commercial soil (COOP, Switzerland) in a climate chamber (23°C, 60% r. h., 16D: 8L, 50’000 lm/m^2). 8-10 day old maize and cowpea and 13-14 day old cotton plants were used for the experiments.

Insects

Spodoptera littoralis (Noctuidae: Lepidoptera) eggs were supplied weekly by Syngenta (Stein, Switzerland). The eggs were incubated in Petri dishes (9 cm diam., 1.5 cm high) on moist filter paper. After emergence larvae were placed on artificial diet in plastic boxes (15x9x5 cm) at room temperature, until they were used for parasitoid rearing as described by Turlings et al. (2004).

For the pre-imaginal learning experiment four different rearing groups were maintained. The parasitized caterpillars were kept in transparent plastic boxes (15 x 13 x 5.5 cm) on either a wheatgerm-based artificial diet or on maize, cotton or cowpea leaves until cocoon formation. Cocoons were separated from the leaves or from artificial diet and kept separated in Petri dishes until adult emergence. Emerging adults of each rearing group were sexed and kept separated in cages Bugdorm-1 (30 x 30 x 30 cm) (MegaView Science Education Services Co. Ltd., Taiwan) at a sex ratio of 1:2 (male: female), with moist cotton wool and honey as a food source.

For the host acceptance experiment the parasitized larvae were only fed with artificial diet.

Parasitized larvae and adults of *C. marginiventris* were held until the experimental day in an incubator (25°C and 16L: 8D). All experiments were conducted with 2-4 days old naïve mated females.

Experiments

Effect of pre-imaginal experience on the responses of adult females to plant induced odors

To test if the innate preferences of the naïve wasps are changed by the diet used to rear the parasitized larvae we carried out an experiment in the same six-arm olfactometer in which naïve *C. marginiventris* females did not show a preference for any of the induced odors produced by the same three plant species, whereas after an oviposition experience on larvae eating on one of the plants, the females preferred the odors of the plant species that they had experienced (Tamò, 2006). The six-arm olfactometer has been described in detail by Turlings et al. (2004).

Three odor sources were prepared the day before an experiment started. The first one consisted of two maize plants, the second of two cotton plants and the third of two cowpea plants. Each pair of plants was placed in an odor source vessel of the olfactometer and fifteen 2nd instar *S. littoralis* larvae were allowed to feed on them during one night. The three other odor source vessels were left empty. For each replicate the odor sources were placed in a different position, but always with one empty chamber between two chambers that contained plants.

On each test day 4 groups of six females were released in the choice chamber and their choices were recorded after 30 min. The four groups of wasps were the following: 1) females from parasitized larvae reared on artificial diet, 2-4) females from parasitized larvae reared on maize, cotton or cowpea leaves. None of the wasps had contacted hosts or their diets as adults. The experiment was replicated 12 times: for each replicate the odor sources were placed in a different position and the order in which the parasitoid group were released was alternated. After an experimental day, all parts of the olfactometer were water washed and rinsed with acetone and hexane. The glass parts were then placed in a drying oven (250°C) overnight. Statistical analyses of the parasitoid choices in the olfactometer were based on the log linear model described in Chapter 3.

Influence of previous oviposition experience on host acceptance

A second experiment was carried out to test if the host acceptance and the parasitization rate were different among naïve and experienced females, and if the diet or plant experienced in previous host contact had an effect on the motivation to parasitize on the same substrate or on a different one.

The day before an experiment in the late afternoon 6 plastic containers (9.5 cm diam., 5 cm high) for each substrate type were prepared. Substrates used were the following: 1) two pieces (10 x 10 x 10 mm) of artificial diet 2-4) a young leaf of maize, cotton or cowpea. Twenty 2nd instar *S. littoralis* larvae were added to each container, allowing them to eat and defecate during overnight. The next day, individual females parasitoids were introduced in the containers to provide them with a contact experience with hosts (two ovipositions) and the associated odors. Four females remained inexperienced (naïve). Females of the experience groups were, immediately after their experience, introduced into a new cup with a certain type of diet and 20 host larvae. For each female a new exposure cup was used. The females were observed in these exposure cups during five minutes. The time elapsed until the first oviposition and the number of oviposition during the five minutes were recorded. To test for differences of these two parameters among naïve and females with a prior experience a GLM model was used. In cases of significance, a pair wise comparison between treatments was performed by the Tukey test.

Results

Effect of pre-imaginal experience on the responses of adult females to plant induced odors

Naïve *C. marginiventris* females had no obvious preference for the induced odors of a particular plant species. Independently of the rearing they were coming from they were always significantly more attracted to the arms containing an odors source comparaed with the empty arms, but did not show a preference for one of the odor sources ($P < 0.001$, Figure 1).

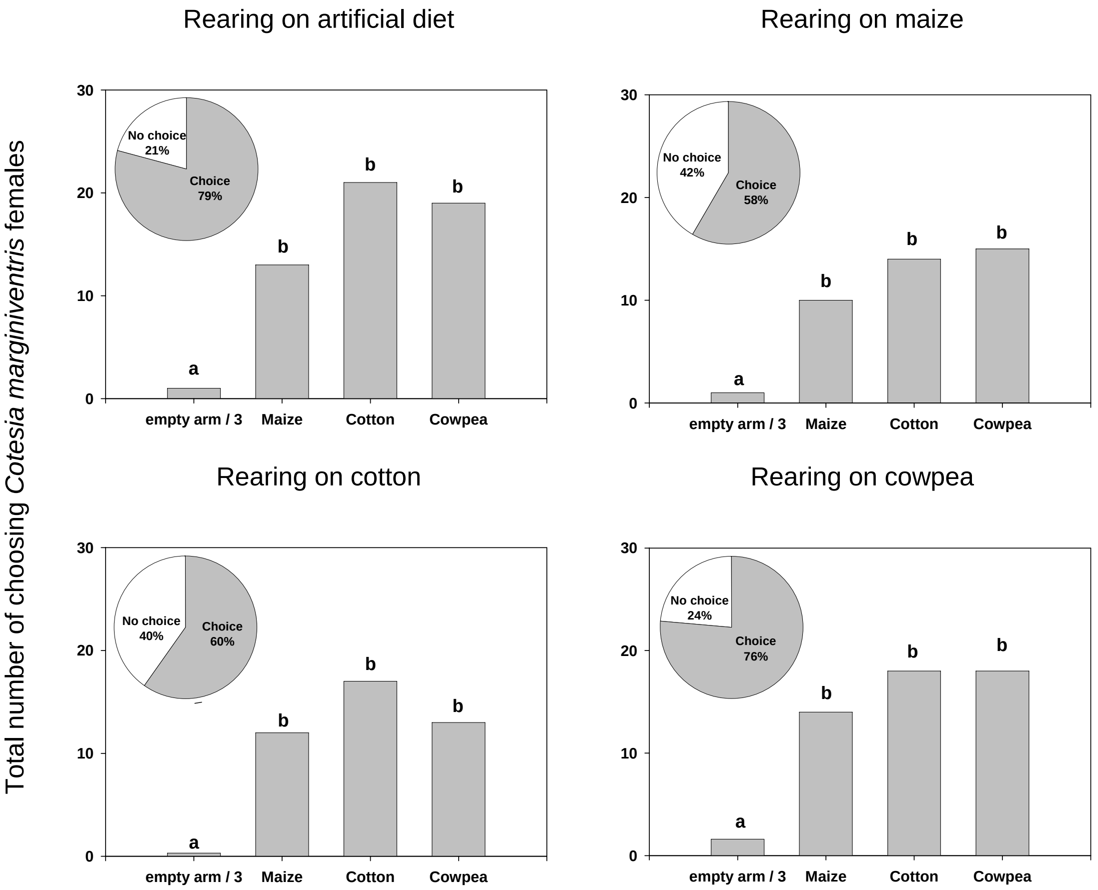


Figure 1. : Olfactometric responses of the parasitoids *Cotesia marginiventris* when given a choice between the odours of *Spodoptera littoralis*-infested maize, cotton and cowpea plants. The wasps were coming either from hosts reared on artificial diet, or on one of the three tested plants. The pie chart shows the percentage of wasps that entered an arm. The bars represent the total number of parasitoid attracted to the arms containing one of the three tested host plants or of parasitoids that went into one of the empty arms. Choices for the empty arms were summed and divided by 3. Bars having no letters in common represent significantly different response levels within each graph (P < 0.001).

Influence of previous oviposition experience on host acceptance

Females with an oviposition experience on one of the three plant species parasitized more larvae than naïve females and than females that had had an ovipostion experience on artificial diet. Naïve females and females with an oviposition experience on one of the three plants species parasitized significantly less larvae on artificial diet, than on the plant species, and no significant difference among plant species was found. The females with an oviposition experience on artificial diet showed no significant differences in the number of larvae that they parasitized on the different diets (P<0.05, Figure 2).

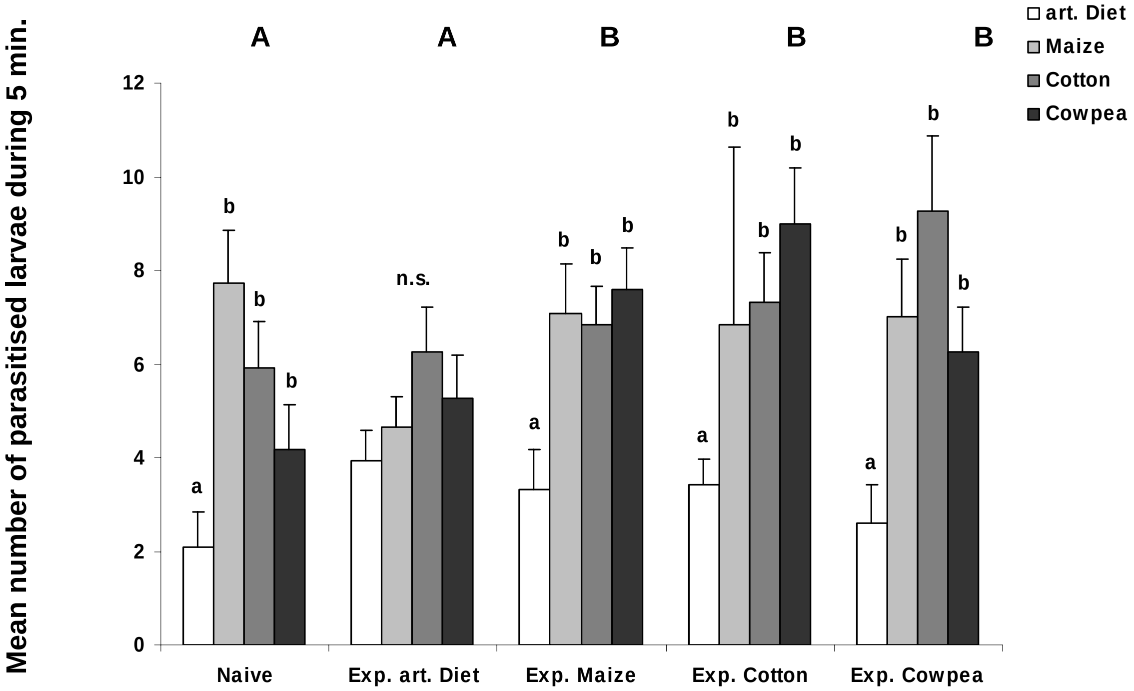


Figure 2 : Mean number of larvae parasitised by *C. marginiventris* females during 5 min. in no choice situation. Different letters indicate significant difference between treatments within each pairs of experience groups . Different capital letters indicate significant difference between experience groups (p < 0.05).

The time needed by the females to start to parasitize was always significantly longer for parasitoids from larvae that had been fed on artificial diet, unless the females parasitoid had a previous contact experience with larvae on artificial diet. For the females with a previous experience on a plant species, no differences in the time until first oviposition were found among the three plant species tested (p<0.05, Figure3).

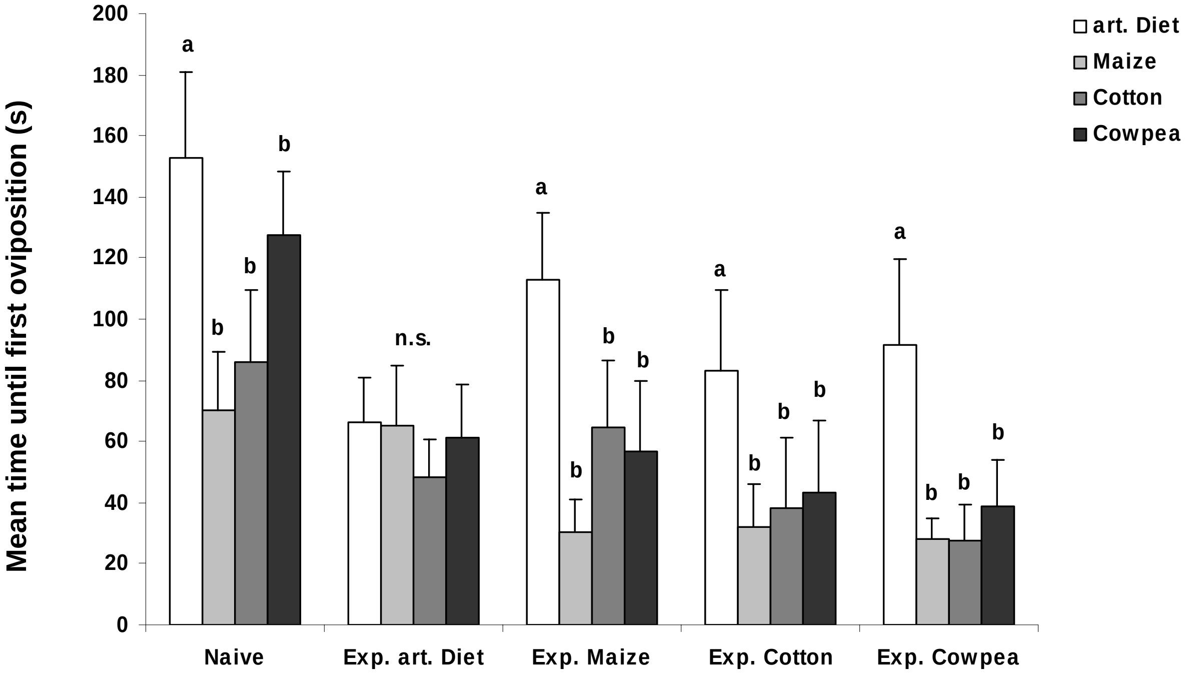


Figure 3 : Mean time needed by *Cotesia marginiventris* females to start to parasitise in no choice situation. Different letters indicate significant difference between treatments within each pairs of experience groups (p < 0.05).

Discussion

Previous studies had shown that experiences as adults affect the responses of *C. marginiventris* females to long-range olfactory cues (Turlings et al., 1990; Turlings et al., 1993; Tamò, 2006). Here we show that pre-imaginal experiences or experiences during emergence have no apparent effect on subsequent olfactory responses. *C. marginiventris* females reared from host larvae that had been feeding on one of three host plants responded similarly in the olfactometer. Females from each treatment were readily attracted to the plant odors, but in no case they did show a preference for a particular odor. Hence, we did not find any evidence for pre-imaginal learning in this parasitoid species. The parasitoids used in this experiment had emerged from cocoons collected and separated before emergence from the diet on which they were spun. For this reason the adult females had no direct contact with the plants upon emergence, but only with host habitat cues that were possibly adsorbed onto their cocoons. Eventhough the majority of the current examples of odor learning at emergence show that the learnt cues are adsorbed on the cocoon (Hérard et al., 1988; Cortesero and Monge, 1994; van Emden et al., 1996; Storeck et al., 2000), we cannot exclude that in the field, conditioning at emergence could take place, whereby *C. marginiventris* females might perceive odors directly coming from a plant species and subsequently develop a preference for the odor of this specific plant. From previous studies (Turlings et al., 1993; Tamò, 2006) that an experience as an adult with a particular plant odor during an encounter with a host causes *C. marginiventris* females to prefer the experienced odor. This type of associative learning by adults has been shown for many other parasitoid species (Vet, 1983; Drost, Lewis and Tumlinson, 1988; Lecomte and Thibout, 1993; Turlings et al., 1993; Bjorksten and Hoffmann, 1995; Geervliet et al., 1998), and some studies show that preferences developed during the preimaginal phase or by emergence can readily be changed as a result of ensuing foraging experience, confirming the notion that learning as adult often outweighs the effect of preimaginal conditioning in parasitoids (Hérard et al., 1988; Turlings et al., 1993; Storeck et al., 2000). Since *C. marginiventris* is an extreme generalist species for both the host and the host plant species, fixing a preference for the plant species on which an individual developed might actually be disadvantageous. That the wasp does not incorporate information from its immature experiences in foraging decisions seems to be confirmed by the fact that freshly emerged naïve *C. marginiventris* adults respond best to odors that are generally associated with damaged plants, such as typical green leafy volatiles, while after an experience the females shift their preferences towards more specific blends containing induced volatiles that differ among plant species (Hoballah and Turlings, 2005; Tamò, 2006).

The experiment on host acceptance revealed that prior oviposition experience had an effect on host acceptance in terms of time until first oviposition and on the subsequent oviposition rate. Females with an oviposition experience on one of the three plant species subsequently parasitized more larvae during five minutes than naïve females and females with an oviposition experience on artificial diet. Naive females and females with an experience on one of the plant species started to parasitize quicker and parasitized more if the larvae were feeding on one of the three plant species compared with the larvae feeding on artificial diet. Interestingly, females with an oviposition experience on artificial diet subsequently parasitized the larvae on artificial diet as readily as the larvae on the plants, possibly a result of an association between host presence and “unfamiliar” substances that are normally not linked with host presence. No differences were found among wasps that had experienced the different plant species, implying that, at short range, plant stimuli in general enhance oviposition drive, but that this is primarily the result of priming (increasing “motivation”) rather than associative learning. These results indicate that plant factors are involved in stimulating oviposition as well as in the priming effect.

It could be expected that rearing on an artificial diet negatively affects a parasitoids responses to natural stimuli, as appears to be the case for *Diachasmimorpha* *longicaudata* a parasitoid of fruitfly larvae. This parasitoid was found to prefer to oviposit in larvae reared on artificial diet than in larvae on their host plant, showing a modified parasitization behavior (Bautista and Harris, 1997). The colony of *C. marginiventris* used in our study has been reared on a wheat based artificial diet for several years, but this does not seem to have altered the attraction of naïve adult females to induced plant odors nor the acceptance by adult females of larvae on a host plant. Naïve females coming from the rearing on artificial diet were attracted to the induced plant odors as readily as the females coming from the rearings on host plants. That the rearing on artificial diet for many generations has not importantly altered the parasitoid's natural responses is also indicated by the fact that they parasitized quicker and more larvae on one of the three plant species than on artificial diet, unless they had a previous experience with artificial diet fed larvae.

The difference in the time needed by a female to start to parasitize between the artificial diet and the three plant species could also be partly explained by visual stimuli that the parasitoids perceived in the testing cups. Several studies have demonstrated that for the host finding and acceptance of the hosts the visual stimuli may play a role in addition to volatiles produced by the plants and kairomones from host larvae and their faeces (McAuslane et al., 1990b, 1991; Tripathi and Singh, 1994; Wäckers and Lewis, 1994; Battaglia et al., 1995). The single leaves and/or the host faeces in the cups with the plant diet might have provided visual recognition cues.

It can be concluded from this laboratory study that the responses of *C. marginiventris* females to host location stimuli are more affected by adult experiences than by preimaginal experiences and that, even if reared on unnatural diets, immature wasps do not miss out on vital information that is needed for effective performance as adults.

Acknowledgements

Thanks are due to all members of the laboratoire d'entomologie évolutive (University of Neuchâtel) for their continuous support and scientific feedback. We specially thank Yves Borcard and Liselore Roelfstra for the parasitoids rearing, Syngenta (Basel, Switzerland) for the weekly shipment of *S. littoralis*, Donald Nordlund for providing *C. marginiventris* and Jacqueline Moret for aid with the statistics.

This research was supported by a grant of the Swiss National Science Foundation (31-058865.99) and by the Swiss National Centre of Competence in Research "Plant Survival".

References

Battaglia, D., Pennacchio, F., Romano, A. and Tranfaglia, A. (1995) The role of physical cues in the regulation of host recognition and acceptance behavior of *Aphidius ervi* Haliday (Hymenoptera, Braconidae). *J. Insect Behav.* **8**: 739-50.

Bautista, R.C. and Harris, E.J. (1997) Effect of insectary rearing on host preference and oviposition behavior of the fruit fly parasitoid *Diachasmimorpha longicaudata*. *Entomol. Exp. Appl.* **83**: 213-18.

Benrey, B., Denno, R.F. and Kaiser, L. (1997) The influence of plant species on attraction and host acceptance in *Cotesia glomerata* (Hymenoptera:Braconidae). *J. Insect Behav.* **10**: 619-30.

Bjorksten, T.A. and Hoffmann, A.A. (1995) Effects of pre-adult and adult experience on host acceptance in choice and non-choice tests in 2 strains of *Trichogramma*. *Entomol. Exp. Appl.* **76**: 49-58.

Bogahawatte, C.N.L. and van Emden, H.F. (1996) The influence of the host plant of diamond-back moth (*Plutella xylostella*) on the plant preferences of its parasitoid *Cotesia plutellae* in Sri Lanka. *Physiol. Entomol.* **21**: 93-96.

Corbet, S.A. (1985) Insect chemosensory responses - a chemical legacy hypothesis. *Ecol. Entomol.* **10**: 143-53.

Cortesero, A.M. and Monge, J.P. (1994) Influence of preemergence experience on response to host and host-plant odors in the larval parasitoid *Eupelmus vuilleti*. *Entomol. Exp. Appl.* **72**: 281-88.

Dicke, M. and Vet, L.E.M. (1999). Plant-carnivore interaction: evolutionary and ecological consequences for plant, herbivore and carnivore. In Olff H., Brown V.K. and Drent R.H. (eds.), *Herbivore: between plants and predators*, Blackwell Science, Oxford, pp. 483-520.

Drost, Y.C., Lewis, W.J. and Tumlinson, J.H. (1988) Beneficial arthropod behavior mediated by airborne semiochemicals.5. Influence of rearing method, host plant, and adult experience on host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis*. *J. Chem. Ecol.* **14**: 1607-16.

Drost, Y.C., Lewis, W.J., Zanen, P.O. and Keller, M.A. (1986) Beneficial arthropod behavior mediated by airborne semiochemicals.1. Flight behavior and influence of preflight handling of *Microplitis croceipes* (Cresson). *J. Chem. Ecol.* **12**: 1247-62.

Duan, J.J. and Messing, R.H. (1999) Effects of origin and experience on patterns of host acceptance by the opiine parasitoid *Diachasmimorpha tryoni*. *Ecol. Entomol.*, **24**: 284-91.

Gandolfi, M., Mattiacci, L. and Dorn, S. (2003) Preimaginal learning determines adult response to chemical stimuli in a parasitic wasp. Proc. Roy. Soc. Lond. B **270**: 2623-29.

Geervliet, J.B.F., Vreugdenhil, A.I., Dicke, M. and Vet, L.E.M. (1998) Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula*. *Entomol. Exp. Appl.* **86**: 241-52.

Hoballah, M.E.F. and Turlings, T.C.J. (2005) The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. *J. Chem. Ecol.* **31**: 2003-2018.

Hérard, F., Keller, M.A., Lewis, W.J. and Tumlinson, J.H. (1988) Beneficial arthropod behavior mediated by airborne semiochemicals.4. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson (Hymenoptera, Braconidae). *J. Chem. Ecol.* **14**: 1597-606.

Lecomte, C. and Thibout, E. (1993) Preimaginal and post-imaginal experience in a specialist parasitoid *Diadromus pulchellus* (Hym, Ichneumonidae). *Entomophaga* **38**: 175-84.

Loke, W.H. and Ashley, T.R. (1984a) Behavioral and biological responses of *Cotesia marginiventris* to kairomones of the Fall Armyworm, *Spodoptera frugiperda*. *J. Chem. Ecol.* **10**: 521-29.

Loke, W.H. and Ashley, T.R. (1984b) Sources of Fall Armyworm, *Spodoptera frugiperda* (Lepidoptera, Noctuidae), kairomones eliciting host-finding behavior in *Cotesia* (=*Apanteles*) *marginiventris* (Hymenoptera, Braconidae). *J. Chem. Ecol.* **10**: 1019-27.

Mattiacci, L., Hutter, E., Schoch, D., Scascighini, N. and Dorn, S. (2000) Plant-odour mediates parasitoid host handling and oviposition in an endophytic tritrophic system. *Chemoecol.* **10**: 185-92.

McAuslane, H.J., Vinson, S.B. and Williams, H.J. (1990a) Effect of host diet on flight behavior of the parasitoid *Campoletis sonorensis* (Hymenoptera, Ichneumonidae). *J. Entomol. Sci.* **25**: 562-70.

McAuslane, H.J., Vinson, S.B. and Williams, H.J. (1990b) Influence of host plant on mate location by the parasitoid *Campoletis sonorensis* (Hymenoptera, Ichneumonidae). *Environm. Entomol.* **19**: 26-31.

McAuslane, H.J., Vinson, S.B. and Williams, H.J. (1991) Stimuli influencing host microhabitat location in the parasitoid *Campoletis sonorensis*. *Entomol. Exp. Appl.* **58**: 267-77.

Monteith, L.G. (1958) Influence of food plant of host on attractiveness of the host to tachinid parasites with notes on preimaginal conditioning. *Canad. Entomol.* **90**: 478-82.

Song, S.J., Bourchier, R.S. and Smith, S.M. (1997) Effect of host diet on acceptance of eastern spruce budworm eggs by *Trichogramma minutum*. *Entomol. Exp. Appl.* **84**: 41-47.

Storeck, A., Poppy, G.M., van Emden, H.F. and Powell, W. (2000) The role of plant chemical cues in determining host preference in the generalist aphid parasitoid *Aphidius colemani*. *Entomol. Exp. Appl.* **97**: 41-46.

Tamò, C. (2006) *A comparative study on the plant odour preferences and learning ability of three solitary endoparasitoids of Spodoptera species*, University of Neuchâtel.

Tripathi, R.N. and Singh, R. (1994) Host-finding and host acceptance behavior of an Aphid Parasitoid *Lysiphlebia mirzai* Shuja-Uddin (Hymenoptera, Aphidiidae). *J. Adv. Zool.* **15**: 37-43.

Turlings, T.C.J. and Benrey, B. (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecosci.* **5**: 321-33.

Turlings, T.C.J., Davison, A.C. and Tamo, C. (2004) A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiol. Entomol.* **29**: 45-55.

Turlings, T.C.J., Tumlinson, J.H. and Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250**: 1251-53.

Turlings, T.C.J., Tumlinson, J.H., Lewis, W.J. and Vet, L.E.M. (1989) Beneficial arthropod behavior mediated by airborne semiochemicals.8. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *J. Insect Behav.* **2**: 217-25.

Turlings, T.C.J., Waeckers, F., Vet, L.E.M., Lewis, W.J. and Tumlinson, J.H. (1993). Learning of host-finding cues by hymenopterous parasitoids. In Papaj D.R. and Lewis A. (eds.), *Insect Learning: Ecological and Evolutionary Perspectives*, Chapman and Hall, New York, pp. 51-78.

Turlings, T.C.J. and Wäckers, F.L. (2004). Recruitment of predators and parasitoids by herbivore-damaged plants. In Cardé R.T. and Millar J. (eds.), *Advances in Insect Chemical Ecology*, Cambridge University Press, pp. 21-75.

van Emden, H.F., Sponagl, B., Baker, T., Ganguly, S. and Douloumpaka, S. (1996) Hopkins 'host selection principle', another nail in its coffin. *Physiol. Entomol.* **21**: 325-28.

Vet, L.E.M. (1983) Host-habitat location through olfactory cues by *Leptopilina clavipes* (Hartig) (Hym, Eucoilidae), a parasitoid of fungivorous Drosophila - the influence of conditioning. *Neth. J. Zool.* **33**: 225-48.

Vet, L.E.M. (1987). The influence of learning on habitat location and acceptance by parasitoids. In INRA (ed.), *Parasitoid insects*, Vol. 48, Les colloques de L'INRA, Lyon, pp. 29-34.

Vet, L.E.M. and Groenewold, A.W. (1990) Semiochemicals and learning in parasitoids. *J. Chem. Ecol.* **16**: 3119-35.

Vet, L.E.M., Lewis, W.J. and Carde, R.T. (1995). Parasitoid foraging and learning. In Cardé R.T. and Bell W.J. (eds.), *Chemical Ecology of Insects 2*, Chapman and Hall, New York, pp. 65-101.

Vet, L.E.M. and Schoonman, G. (1988) The influence of previous foraging experience on microhabitat acceptance in *Leptopilina heterotoma*. *J. Insect Behav.* **1**: 387-92.

Vinson, S.B. (1998) The general host selection behavior of parasitoid hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biol. Control* **11**: 79-96.

Wickremasinghe, M.G.V. and van Emden, H.F. (1992) Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. *Physiol. Entomol.* **17**: 297-304.

Wäckers, F.L. and Lewis, W.J. (1994) Olfactory and visual learning and their combined influence on host site location by the parasitoid *Microplitis croceipes* (Cresson). *Biol. Control* **4**: 105-12.

Chapter 5

Relative importance of host derived and plant-derived volatiles in the attraction of the larval parasitoids *Cotesia marginiventris* and *Microplitis rufiventris*

Based on: Cristina Tamò, Matthias Held, Maria Elena Hoballah and Ted C. J. Turlings. Relative importance of host derived and plant-derived volatiles in the attraction of the larval parasitoids *Cotesia marginiventris* and *Microplitis rufiventris.* In preparation for submission to *Journal of Chemical Ecology*.

Abstract

Using a six-arm olfactometer, we compared the responses of the two larval endoparasitoids, *Cotesia marginiventris* and *Microplitis rufiventris*, to the odors of maize plants that were induced to emit volatiles. Naïve and wasps that had had an oviposition experience on an host-damaged maize plant were offered the choice between undamaged maize plants, undamaged plants treated with caterpillar regurgitant, freshly damaged plants (scratched and treated with caterpillar regurgitant), plants with the same but older damage, plants with an old and a fresh damage and plants actively fed on by herbivores. The results revealed that naïve *C. marginiventris* are highly responsive to freshly damaged plants (which typically release green leafy volatiles) and become more responsive to plants with older damage that release specific herbivore-induced volatiles after experience. In contrast, naïve *M. rufiventris* respond best to the complete blend of plant-produced volatiles, while experience rendered this wasp more responsive to volatiles that are directly associated with the herbivores and/or their by-products. Although both wasps have similar host plant ranges, they clearly employ different strategies to find their hosts.

Introduction

Natural enemies of herbivores make use of the volatiles produced by plants under herbivores attack to locate their prey or hosts (for reviews see Vet and Dicke, 1992; Turlings and Benrey, 1998; Dicke, 1999; Turlings and Wäckers, 2004). Several parasitoid species have been shown to be differently attracted to the odors produced by different plant species (Drost et al., 1988; Turlings et al., 1989; Turlings et al., 1990a; McAuslane et al., 1991; Geervliet et al., 1996; De Moraes and Lewis, 1999; Tamò et al., 2006) and also by different varieties of the same plant species (Elzen et al., 1986; Takabayashi et al., 1991; Loughrin et al., 1995; Rapusas et al., 1996; Geervliet et al., 1997; Hoballah et al., 2002). Analyses of the induced volatiles produced by different maize varieties and some of their wild ancestors have revealed considerable differences in the quantity as well as the quality (relative ratios) of the produced volatiles (Gouinguene et al., 2001; Hoballah et al., 2002; Degen et al., 2004). We also found that these differences are reflected in the attractiveness of the maize varieties for parasitoids, which was not only due to the differences in quantity, but also to qualitatively differences (Hoballah et al., 2002). The behavior of various parasitoids can also be affected by their experiences with host location cues, naïve wasps and wasps that have experienced certainly plant odors while contacting hosts are often differently attracted to different plant species or varieties (Turlings et al., 1989; Eller et al., 1992; McCall et al., 1993; Geervliet et al., 1998).

Most plants under herbivore attack typically release so-called green-leaf volatiles immediately upon damage and release various terpenoids, phenolic and nitrogenous compounds only after they have been damaged for some time (Dicke 1999b; Paré and Tumlinson, 1999; D'alessandro and Turlings, 2006).

The green-leaf volatiles (which are indicative of fresh damage) are very similar for all plant species, whereas the blend of the truly induced volatiles varies between plant species and even between different genotypes of the same species (Loughrin et al., 1995; Gouinguene et al., 2001; Krips et al., 2001; Degen et al., 2004). Studies on the antennal perception of volatiles using electroantennogramm (EAG) recording showed that generally parasitoids perceive most plant volatiles (Baehrecke et al., 1989; Ramachandran and Norris, 1991; Li et al., 1992; Whitman and Eller, 1992; Du et al., 1998; Gouinguene et al., 2005). However, EAG responses do not necessarily reflect central integration of peripheral stimuli and therefore do not necessarily provide information on the resulting behavioral responses. Nevertheless, assays that have tested the actual attraction of parasitoids to specific compounds, indicate that many plant volatiles are indeed attractive for parasitoids (Elzen et al., 1984; Whitman and Eller, 1992; Vaughn et al., 1996; Du et al., 1998). Yet, it remains unclear which of the compounds are essential for attraction (D'Alessandro and Turlings, 2005, 2006).

The release of volatiles by maize plants in response to caterpillar damage has been studied extensively. In maize, only green leaf volatiles are emitted by the plant immediately upon damage, while various terpenoids and the aromatic compound indole start to be emitted some hours after damage (Turlings et al., 1998). As long as the caterpillars continue to feed, the green leaf volatiles will be released. A one-time mechanical damage applied to maize leaves also results in the emission of green leaf volatiles, which ceases within hours (Turlings et al., 1998). Applying caterpillar regurgitant to a mechanically damaged site causes a maize plant to systemically emit the terpenoids and indole in amounts and ratios that are similar to those emitted by plant damaged by caterpillars (Turlings et al., 1990b; Turlings and Tumlinson, 1992; Turlings et al., 1998). This provides the possibility to treat maize plants in a specific way and to have them release groups of compounds that are typical for fresh and old damage (Degen et al., 2004).

Using this approach, Hoballah and Turlings (2005) tested the role of fresh versus old damage in the attraction of the two species *C. marginiventris* and *M. rufiventris*, using both naïve and experienced females. The wasps were offered the choice between three different mechanically damaged maize plants, thus producing three different blends, the first one composed of almost only green leaf volatiles, the second one with a mixture of green leaf volatiles and terpenoids plus indole and the third one composed only of terpenoids plus indole. *C. marginiventris* were found to prefer blends containing green leaf volatiles when naïve and to switch their preferences for blends containing both, green leaf volatiles and terpenoids, after having experienced these compounds while contacting host larvae. In contrast, naïve *M. rufiventris* did not show a preference when they were offered the three blends, and surprisingly exhibited a much weaker response to the offered odors after experience. Since some parasitoid species, like for example *Microplitis croceipes* (Cortesero et al., 1997) and *Cotesia rubecula* (Agelopoulos and Keller, 1994a), have been found to also strongly rely on the odors of the frass of their hosts for host habitat location, the authors hypothesized that the weak response of experienced *M. rufiventris* females was due to the absence of cues that are directly associated with host larvae and/or of their frass. The current study was conducted to test this hypothesis and to specifically compare the role of plant cues versus host cues for *M. rufiventris* and *C. marginiventris*.

Using maize plants as odor sources, we tested the relative attractiveness of the volatiles produced by differently mechanically damaged plants as well as the attractiveness of plants that were actively fed upon by host larvae. Hence, the aim of the present study was to determine if the presence the herbivores play a role in the attraction of *C. marginiventris* and *M. rufiventris* to damaged plants.

Materials and methods

Insects

Hosts

The *S. littoralis* eggs, provided weekly by Syngenta (Stein, Switzerland), were kept in Petri dishes on humidified filter paper in an incubator (25°C and 16L: 8D) until emergence of the larvae. The larvae were then transferred inside clear plastic containers (15 x 13 x 5.5 cm) and fed with a wheat germ-based artificial diet (changed every 1-3 days) at room temperature (19-23°C), until using them either for rearing or for the experiments.

Parasitoids

The *C. marginiventris* population originated from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi, USA). The *M. rufiventris* colony was established in 2000 from individuals collected from parasitized *S. littoralis* larvae in fields in Egypt (Alexandria). Both parasitoids were reared in plastic containers (9.5 cm diam., 5 cm high) on *Spodoptera littoralis* (Noctuidae: Lepidoptera). For the rearing, two 4-7 days old mated females of each parasitoid species were placed for 3-4 h in the presence of 25 *S. littoralis* caterpillars. The parasitized caterpillars were then maintained in clear plastic boxes (15 x 13 x 5.5 cm). All larval stages were fed with wheat germ-based artificial diet until cocoon formation. Cocoons were kept in Petri dishes until adult emergence. Emerging adults were sexed and kept in cages (30 x 30 x 30 cm) (MegaView Science Education Services Co. Ltd., Taiwan) at a sex ratio of 1:2 (male: female). Adult wasps were fed with honey and provided with moist cotton wool as water source. Parasitized larvae and adults of *C.*

marginiventris were held until the experimental day in the above-mentioned incubator, whereas parasitized larvae and adults of *M. rufiventris* were held in the laboratory under ambient light and temperature conditions (19-23°C). All the parasitoid females used for the experiments were 2-4 day old.

Plants

Maize plants (*Zea mays*, var. Delprim) were grown in a climate chamber (23°C, 60% r. h., 16D: 8L, 50'000 lm/m^2). Two seeds were planted per pot (200ml) in fertilized commercial soil (COOP, Switzerland). Plants were used in the experiments when they were 8-10 days old. The day before an experiment, six arm olfactometer pots (250 ml, 4.5 cm diameter, 11 cm high), each with two plants, were prepared and treated as described below.

Experimental design

The six arm olfactometer has been described by Turlings et al. (2004). Each arm was connected to a pot that contained two maize plants that had received one of the following treatments: 1) the plants were left unarmed, 2) without damaging the leaves10 µl of caterpillar regurgitant was smeared on the underside of two leaves of each plant 0.5h before starting the actual bioassay 3) the underside of two leaves of each plant was scratched with a razor blade over an area of 2-4 cm^2 on both sides of the central vein, and 10 µl of caterpillar regurgitant were applied on each damaged site the evening before the experiments (old damage) 4) the plants were treated exactly in the same way as in treatment 3, but just 0.5h before starting the bioassays (fresh damage) 5) the plants were treated in the same way twice, once the evening before the experiments and once 0.5 h before, each time half of the area of the leaves was damaged and half of the caterpillar regurgitant was applied (old + fresh damage) 6) ten 2nd instars *S. littoralis* larvae were placed on the plants in the evening before the bioassay, allowing them to feed overnight (the larvae remained on the plants also during the bioassay). All the plants were left in the dark inside the vessels of the olfactometer overnight and they were illuminated with 10 neon tubes (5 Osram 18W/21-810, alternated with 5 Sylvania Gro-Lux F18W/GRO-T8) of the olfactometer starting 1.5h before and during the bioassay. For each replicate the odor sources were placed in a different position and the experiment was replicated six times for each parasitoid species. On each experimental day three groups of 6 naïve wasps and three groups of 6 experienced females were released alternately. The wasps' choices were determined 30 minutes after release.

To obtain experienced females they were allowed to parasitize twice in a plastic box (9.5 cm diam., 5 cm high) containing one leaf of a maize seedling that had been fed upon by fifteen 2nd instar *S. littoralis* larvae during one night. The experiences occurred just before releasing the wasps in the olfactometer.

Volatile collection

During all experiments 50% of the air passing over the odor sources was pulled through a trapping filter for 3.5h. Volatiles emitted by the maize plants in the vessels of the olfactometer were trapped as described by Turlings et al. (1998). Trapping filters consisted of 7cm glass tubes in which 25 mg of 80-100 mesh Super Q adsorbent (Altech, Deerfiedl, Illinois) was placed and kept in place by two fine mesh metal screens as described by Heath and Manukian (1992). In all experiments, one filter was attached to the horizontal port at the top of each odor source vessel. Before each experiment, the traps were rinsed five times with 200 µl methylene chloride. The first wasp release started 0.5h after a collection started. After each collection, the traps were removed, extracted and analyzed as described below.

Chemical analyses

Immediately after each experiment, the volatiles collected on these filters were extracted with 150 µl of methylene chloride and two internal standards (n-octane and nonly acetate, each 200 ng in 10 µl methylene chloride) were added to the samples. The samples were either analyzed immediately or stored at -70°C for later analysis. For the analysis of each sample, an aliquot of 3 µl was injected on column with the use of an automated injection system onto an apolar HP-1 capillary column (30 m, 0.25 mm I.D., 0.25 μm film thickness). The column was housed in a Hewlett Packard model HP 6890 gas chromatograph equipped with a flame ionisation detector. The oven was held at 50 °C for 3 min and then programmed at 8 °C /min to 230 °C, where it was maintained for 9.5 minutes. The column was preceded by a deactivated retention gap (10m, 0.25mm I.D., Connex, USA) and a deactivated precolumn (30cm, 0.530mm I.D., Connex, USA). Helium (24 cm/s) was used as carrier gas. HP GC Chemstation software was used to quantify all major components based on the known quantity of internal standards.

Initial identification of most compounds was based on comparisons of retention times from previous studies (Bernasconi et al., 1998; Turlings et al., 1998). Identities were confirmed with the mass spectrometry analysis of some samples, using the same column and temperature program (Agilent 5973, transfer line 230°C, source 230°C, quadrupole 150°C, ionisation potential 70 eV, scan range 0-400 amu). The amounts of the 23 dominating compounds in the collections were determined based on their relative peak areas and those of the internal standards.

Statistical treatment of the data

Analyses of the wasp choice data were based on a log-linear model fitted for the expected distribution of the wasps within the olfactometer described by Turlings et al. (2004). We tested for quantitative differences in the total odor emissions as well as for the emissions for individual compounds. A two way analysis of variance was performed to test for differences between the 2 species data sets. Differences in the total amount of produced volatiles between treatments were determined using one way analysis of variance. The Student-Newman-Keuls test was performed for multiple comparisons.

Twenty-three compounds were used for comparison between treatments:). (Z)-3-hexenal; (*E*)-2-hexenal; (*Z*)-3-hexen-(1)-ol; (E)-2-hexen-(1)-ol; (Z)-2-Penten-(1)-ol-acetate; β-myrcene; (*Z*)-3-hexen-(1)-ol acetate; (E)-2-hexen-(1)-ol acetate; linalool; (*E*)-4,8-dimethyl-1,3,7-nonatriene; benzyl acetate; phenethyl acetate; 1-H-indole; benzoic acid, 2-amino-methylester; geranyl acetate; unknown sesquiterpene; (*E*)- β-caryophyllene; (*E*)-α-bergamotene; (*E*)- β-farnesene; α-zingiberene; bisabolene; β-sesquiphellandrene; (E)-nerolidol

To test for differences between treatments for each single compound a Kruskal-Wallis one way analysis of variance followed by a Tukey test for multiple comparisons were performed.

Additionally, to explore the association between plant treatments and the two treatment factors, wasps responses (naïve and experienced) and induced volatiles groups we subjected the data to a principal component analysis (PCA) using the program CANOCO 4.5.

Results

Wasps behavior (Figure 1)

Naïve and experienced females of both species were least attracted to the odors of the undamaged plants (whether treated with regurgitant or not). Naïve *C. marginiventris* were significantly more attracted to the odor sources that had freshly damaged plants and to the maize plants that were being damaged by *S. littoralis* larvae (Fig. 1, A1). After conditioning, they showed a preference for the odor of plants treated twice (fresh and old damage) and plants being damaged by the larvae (Fig. 1, A2).

In contrast, naïve *M. rufiventris* were mostly attracted to plants that were treated twice (old and fresh damage) and to plants with larvae feeding on them (Fig. 1, B1). After experience, they showed a strong preference for the odor of plants with larvae and strongly reduced attraction to the other sources (Fig. 1, B2).

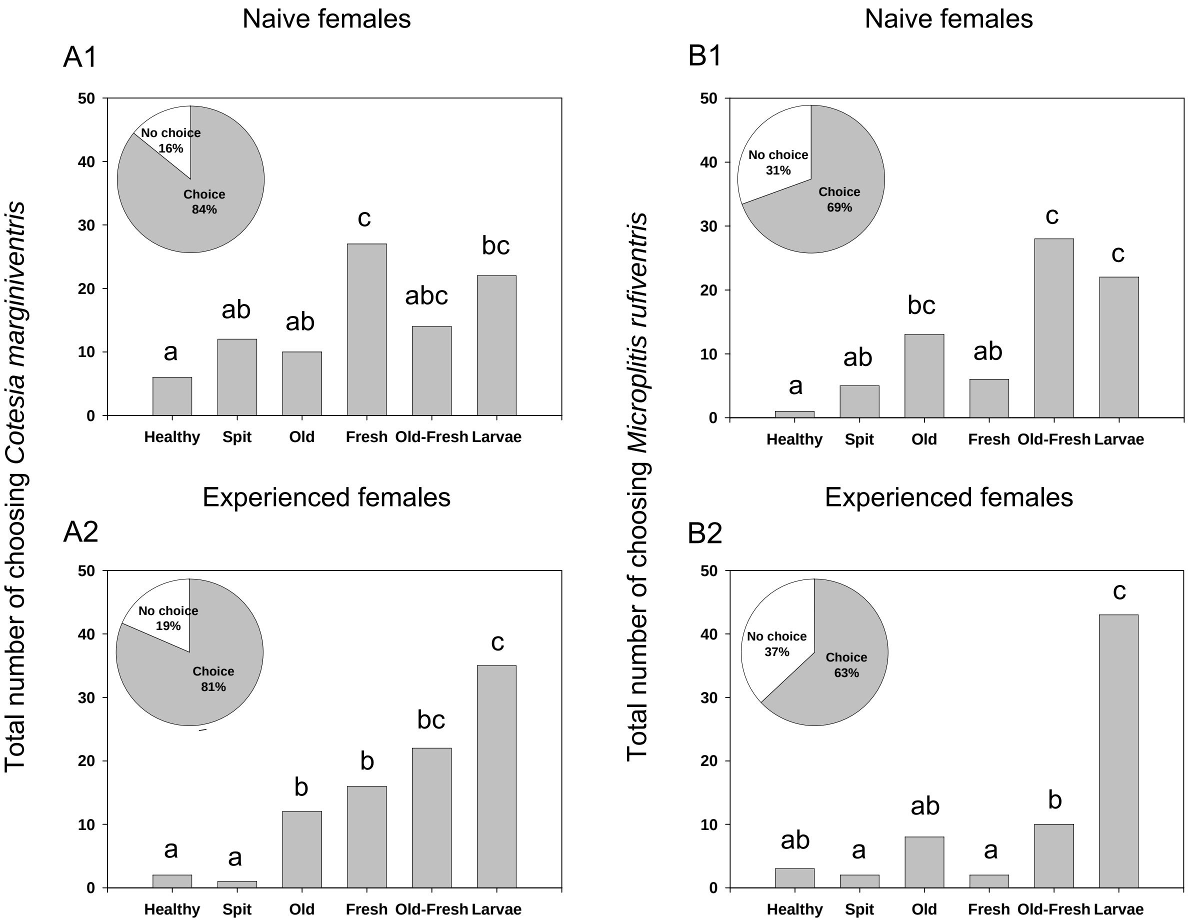


Figure 1.: Total number of naive and experienced *Cotesia marginiventris* and *Microplitis rufiventris* females that were attracted to a specific source in a 6-arm olfactometer. Healthy: two healthy maize plants, Spit: two healthy maize plants with 10 μl of *Spodoptera littoralis* regurgitant on the leaves, Old: two maize plants treated 12 h before experiment started, Fresh: two maize plants treated 30 min before experiment started, Old-Fresh: two maize plants treated 12 h and 30 min before experiment started, Larvae: two maize plants actively fed by 10 *S. littoralis* larvae since 12 h. The pie chart shows the percentage of wasps that entered an arm. The bars represent the total number of parasitoid attracted to one of the 6 arms. Different letters above bars indicate significant differences among total numbers of wasp that chose a particular odor source (A1: P<0.01; A2: P<0.001; B1: P<0.001; B2: P<0.001).

Odor emissions

For the odor emissions we did not find any significant difference between the data sets for the two parasitoid species (P = 0.289), therefore they were combined for the overall comparison between plant treatments. The total amount of volatiles emitted during 3.5 h of collection was considerably different between the different treatments (Figure 2). Twenty-three compounds were detected and consistently found for the damage treatments (Figure 3). As expected the fresh treatment resulted in the emission of mainly green leaf volatiles and the plants with older damage (artificially and insect inflicted) emitted the typically induced compounds (terpenoids and aromatics). There was a significant tendency for the caterpillar damaged plants to emit more than the plants with fresh and old artificial damage, but there was only a significant difference in the emission of indole.

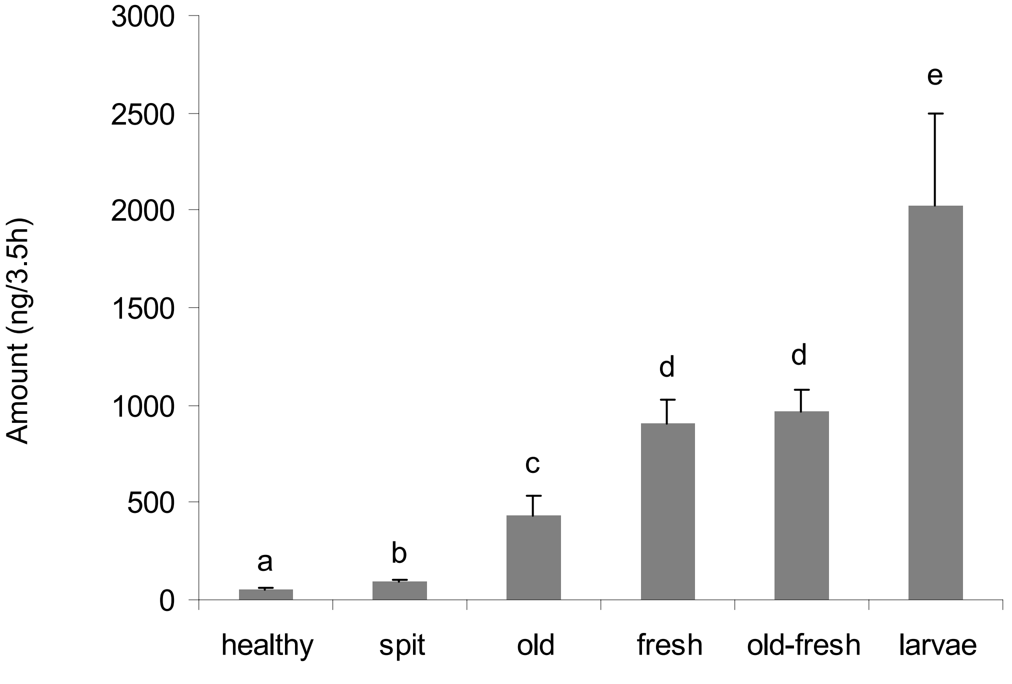


Figure 2.: Mean total amount (±SE) of induced volatiles emitted by maize plants submitted to different treatments, undamaged plants (healthy.), undamaged plants with caterpillar regurgitant (spit), plants treated the evening before the begin of the experiment (old), plants treated 0.5 h before the begin of the experiment (fresh), plants treated the evening before and 0.5 h before the begin of the experiment (old-fresh), plants actively fed by *S. littoralis* larvae (larvae). Letters above each bar indicate significant differences after Student-Newman-Keuls post hoc test for α= 0.05.

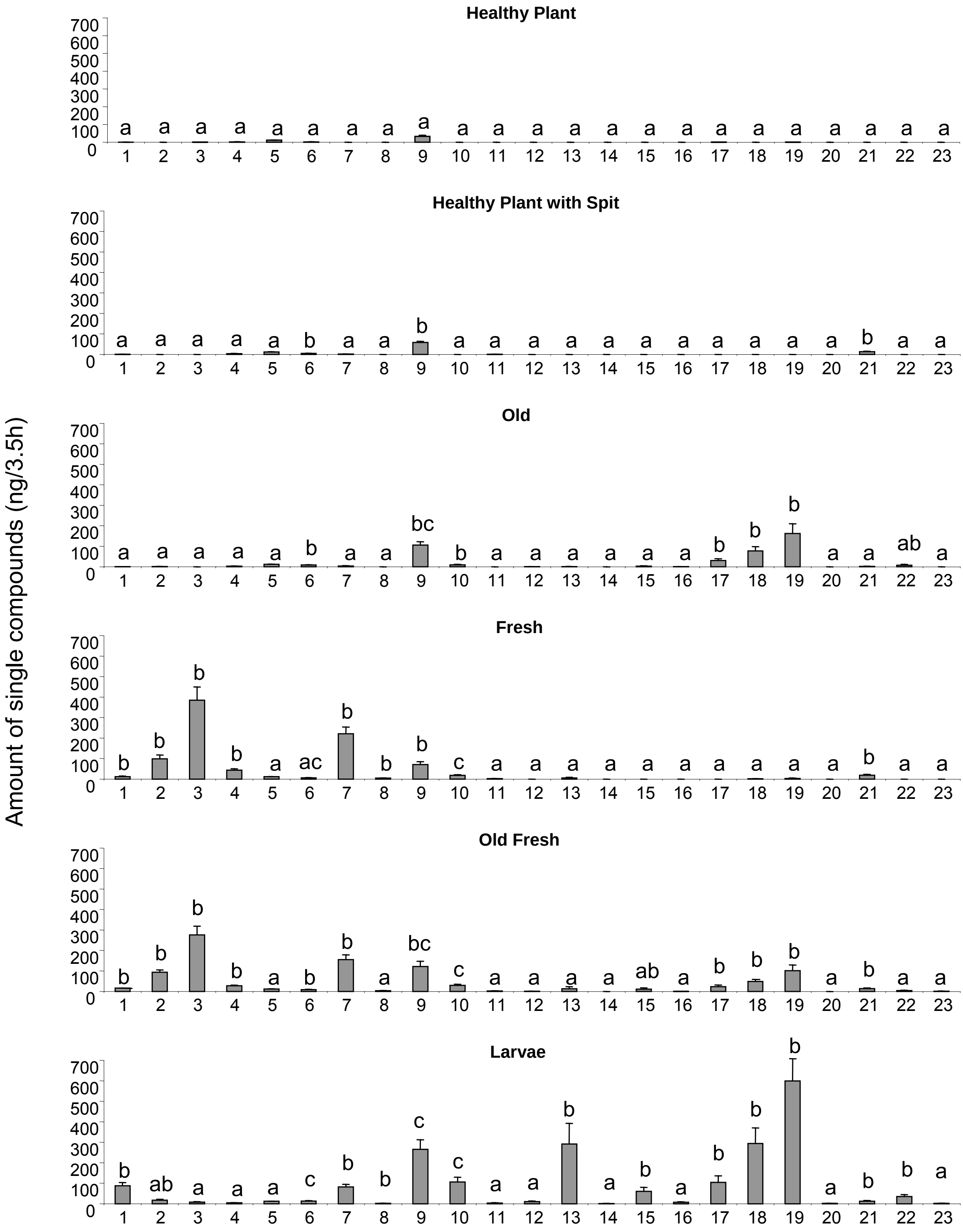


Figure 2. Mean (±SE) amounts of major single volatile compounds emitted during 3.5 hours from two maize plants treated in the different ways elucidated in text. Different letters above bars indicate significant differences in amount of single compounds collected among treatments and *ns* indicate no significant difference among treatments (α=0.05). **(1)** (Z)-3-hexenal; (**2)** (*E*)-2-hexenal; **(3)** (*Z*)-3-hexen-(1)-ol; **(4)** (E)-2-hexen-(1)-ol; **(5)** (Z)-2-Penten-(1)-ol-acetate; **(6)**, β-myrcene; **(7)**; (*Z*)-3-hexen-(1)-ol acetate; **(8)** (E)-2-hexen-(1)-ol acetate; **(9)** linalool; **(10)** (*E*)-4,8-dimethyl-1,3,7-nonatriene; **(11)** benzyl acetate; **(12)** phenethyl acetate; **(13)** 1-H-indole; **(14)** benzoic acid, 2-amino-methylester; **(15)** geranyl acetate; **(16)** unknown sesquiterpene; **(17)** (*E*)- β-caryophyllene;**(18)** (*E*)-α-bergamotene; **(19)** (*E*)- β-farnesene; **(20)** α-zingiberene; **(21)** bisabolene; **(22)** β-sesquiphellandrene; **(23)** (E)-nerolidol

The PCA confirmed that green leaf volatile emissions is positively linked with fresh damage and the other induced volatiles groups are positively linked with older damage (Figure 4a and 4b). The effect of experience could also be visually depicted by these analyses. The insertion of the vectors for the wasps responses indicated that the responses of naïve *C. marginiventris* are highly positively linked to green leaf volatiles while the responses of experienced *C. marginiventris* are positively linked to the other volatiles groups (Figure 4a). The responses of naïve *M. rufiventris* resulted to be positively linked to all volatiles groups, while the responses of experienced *M. rufiventris* resulted to be more positively linked to the induced volatiles groups, in particular the aromatics (Figure 4b). The high eigenvalues indicate that for both wasps species these variables explain more than 80% of the variability.

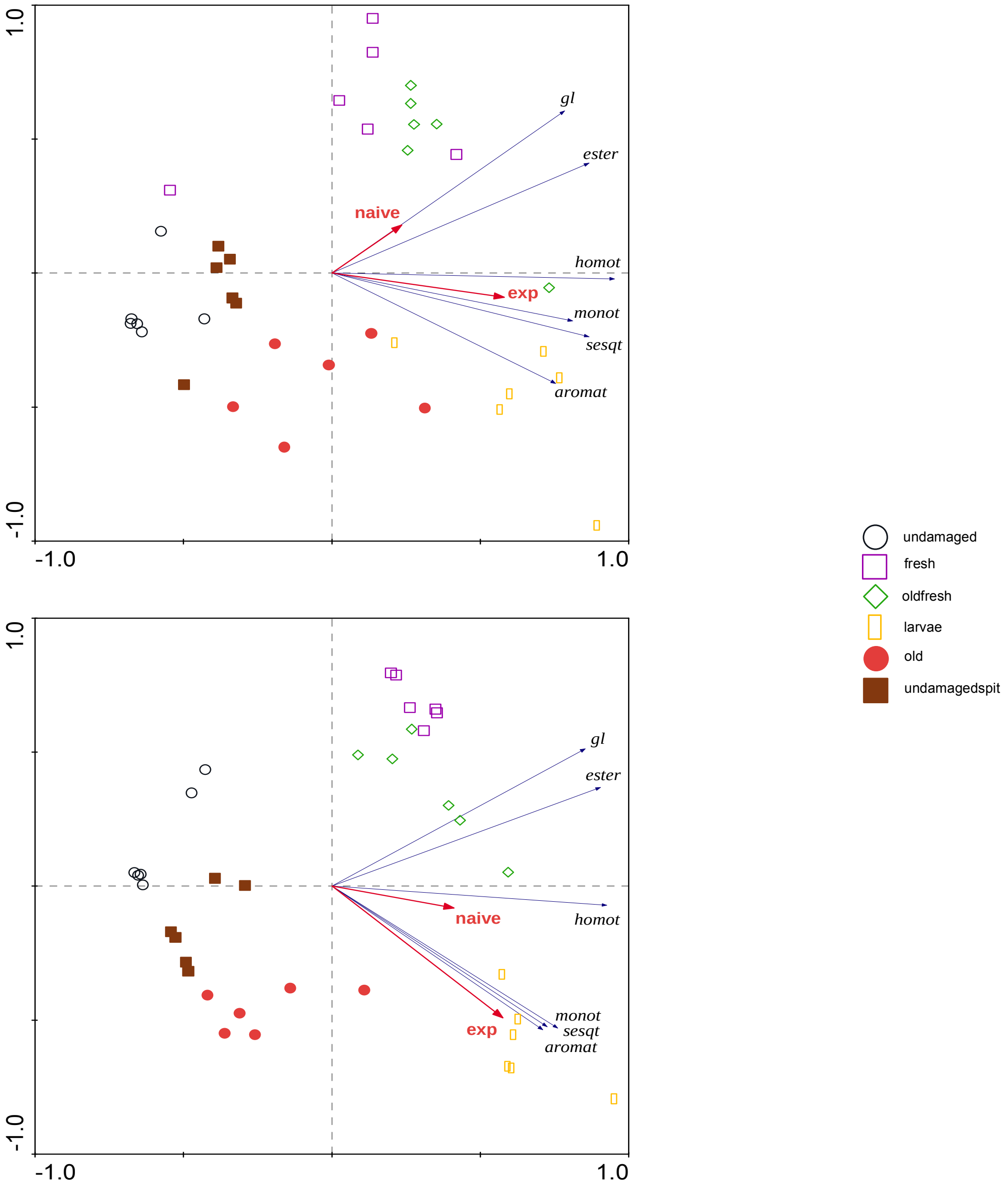


Figure 4. Distribution of induced volatiles groups (plotted as vectors) released by maize plants belonging to the different treatment groups. The vectors naïve and exp indicates the (a) *C. marginiventris* and (b) *M. rufiventris* females responses. (a) Eigenvalues axis 1 (EV = 0.55) and axis 2 (EV = 0.26), (b) Eigenvalues axis 1 (EV = 0.63) and axis 2 (EV = 0.18). Volatiles group abbreviations: gl, green leaf volatiles; ester, esters(acetates); homot, homoterpenes; aromat, N-aromatics; monot, monoterpenoids; sesqu, sesquiterpenoids.

Discussion

The present study confirms that, despite clear similarities in biology and host range, the two parasitoid species *C. marginiventris* and *M. rufiventris* are very different in how they make use of volatile cues to locate hosts, especially in how they adapt their responses to such cues after experiencing them in association with hosts.

Some of these differences were already evident from two earlier studies (Hoballah and Turlings, 2005; Tamò et al., 2006), but here we specifically demonstrate that *M. rufiventris*, after contacting hosts on a host-damaged plant, is most attracted to cues that are closely linked with host presence.

This latter result is surprising as it is commonly expected that plant odors are the key attractants for generalist parasitoids of herbivores (Vet and Dicke, 1992; Turlings and Wäckers, 2004) and that these plant-provided cues are specifically learned during an encounter with hosts on plants (Turlings et al., 1993; Vet et al., 1995). In all the experiments, undamaged maize plants with or without some larval regurgitant applied to the leaves were the least attractive for naïve or experienced females. The relative importance for the attraction of cues associated with fresh damage (mainly green leaf volatiles), cues associated with older damage (mainly terpenoids and some aromatics) and cues coming directly from the larvae and/or the larval frass was found to be different for the two parasitoid species (Figure 1). There were clear differences before and after experience, but also in how experience affected the preferences. Naïve *C. marginiventris* females preferred the odor of plants that were freshly damaged over all the other odors, whereas after experience their preferences switched to odors of a combination of fresh and old damage. This combination is what they experience on a plant that has been damaged by host larvae for over 12h and on which the larvae were still feeding. The experience that they were given is what can be expected during an encounter with hosts on a maize plant in the filed. It should be noted, however, that if they had received an experience with only fresh damage, they would have probably been reinforced to prefer the odor of fresh damage.

Naïve *M. rufiventris* equally preferred the two blends containing both green leaf volatiles and terpenes, while after an oviposition experience the big majority of the females choose the odors produced by the maize plant actively fed on by the larvae. This result confirms the hypothesis proposed by Hoballah and Turlings (2005) that this wasps, during the oviposition experience, learns cues that are directly associated with the hosts and that they actually start avoiding odor blends that do not contain these host cues. Indeed, if the wasps are offered only the odor of artificially treated plants (without host cues), their responses to such an odor significantly drops after experiencing the natural odor of hosts on plants (Hoballah and Turlings, 2005). Again, a different type of experience might have resulted in a different preference, although it is clear that *M. rufiventris* is less inclined to show classical associative learning than *C. marginiventris* (Tamò et al., 2006).

It appears that before experiencing typically induced plant volatiles *C. marginiventris* primarily relies on very general plant cues, such as green leafy volatiles, that are commonly released by many plant species when damaged. After experiencing hosts while perceiving the typical induced odor on a specific plant, females of this species start to use these induced cues in their subsequent foraging efforts. This strategy can be expected to lead them to more hosts on individuals of the same plant species in the vicinity (Turlings et al., 1993). The experienced *C. marginiventris* preferred the blends containing both green leaf volatiles and terpenoids to the blends containing only terpenoids. We could speculate that such a preference allows the experienced females to locate plants of the appropriate species with fresh damage that indicates current presence of herbivores. Experienced *C. marginiventris* females did not distinguish between plants with artificially inflicted old and fresh damage and plants that were being damaged by the herbivores, implying that they fully rely on plant signals and that the presence of the caterpillar and their by-products does not play an important role for the attraction of this species.

In clear contrast, *M. rufiventris* seems to use another strategy, it mostly relies on plant odors when naïve, but after encountering hosts on a plant it starts to use cues that are more specifically associated with host presence. It remains to be determined if these cues come directly from the host larvae and/or their faeces, or even if they use plant cues that cannot be elicited with the artificial treatment. Our analyses of the collected odors did not show any major qualitative differences between the artificially damaged plants with fresh and old damage and the caterpillar damaged plants, except for the compound 1-H-Indole that was released in dramatically higher quantity by the plant actively fed by larvae (Figure 3). The principal component analyses may indeed suggest an importance for aromatic compounds like indole in the attraction of experienced *M. rufiventris* (Figure 4b). Yet, the dramatic reduction of attraction to artificially induced plant odor (Hoballah and Turlings, 2005), seems to suggest that the compounds that are not attractive to experienced *M. rufiventris* are not plant-derived, but this needs to be confirmed.

Based on ample evidence (Elzen et al., 1987; Turlings et al., 1991; McCall et al., 1993; Steinberg et al., 1993) it is generally assumed that induced plant signals are the most important foraging cues for generalist parasitoids of herbivores than cues that come directly from the hosts. Still, there are also a number of studies have shown short and long-range attraction to host frass (Vinson, 1984; Lewis and Tumlinson, 1988; Nordlund et al., 1988; Whitman, 1988a; Whitman, 1988b). Jones et al. (1971) were the first to isolate and identify a compound present in the feces and larvae of *Heliothis zea* that was found by Lewis and Jones (1971) to be responsible for short-range host-seeking responses of the parasite *Microplitis croceipes*. Four larval frass volatiles of *Plutella xylostella* have been identified by Auger et al. (1989) and the parasitoid *Cotesia plutellae* was found to respond significantly to all of them in a Y olfactometer by Reddy et al., (2002). *Microplitis demolitor* females are attracted to volatiles from the larval frass of their hosts *Pseudoplusia includens* in an olfactometer. The major components of the frass volatiles were found to be plant chemicals and the faeces produced on an artificial diet was not attractive to the parasitoids (Ramachandran et al., 1991). Similarly, Agelopoulos and Keller (1994b) found that the main volatiles emitted by frass from *Pieris rapae* and *Plutella xylostella* feeding on cabbage are plant compounds. Frass of both these lepidopteran species is attractive to the parasitoid

Cotesia rubecula, even though *P. xylostella* is not a host. Ramachandran and Norris (1991) propose that the herbivores frass could be a source of 7-carbon volatiles, which might be very important for the attraction of parasitoids. They found high sensitivity of *M. demolitor* antenna to 7-carbon versus 6-carbon compounds, and a heptanoic acid was found for example by Hendry et al. (1973) in the frass of the potato tuber moth.

Odors emitted by damaged plants are expected to be much more readily perceived by parasitoids than odors from host frass because of the much larger quantities of volatiles emitted by plants (Vet and Dicke, 1992), but plant odors may provide limited information on the identity of the herbivore that has been damaging the plants. This problem is partially solved by the ability of parasitoids to learn odors by association (Vet and Groenewold, 1990; Turlings et al., 1993). From a previous study we know that *C. marginiventris* can learn the odors of the plant species maize, cotton and cowpea, while *M. rufiventris* did not show an effect of associative learning in the same experiments (Tamò, 2006). Here we show that *M. rufiventris* likely relies more on the specific odors coming from the feces of its hosts and specifically “learns” to use such cues after perceiving them during an encounter with hosts. This means that these two parasitoid species, even if they are both generalist solitary endoparasitoid with very similar host-plant ranges, they have evolved two entirely different strategies to find their hosts. Next to our current efforts to determine which plant volatiles are key to the attraction of *C. marginiventris* (D’Alessandro and Turlings, 2005; Schnee et al., 2006) will be to isolate and identify the volatiles present in the faeces of *S. littoralis* that are responsible for the “learned” attraction of *M. rufiventris*.

Acknowledgements

Thanks are due to Yves Borcard for the parasitoid rearing. Parasitoids with which we started our lab colonies, were supplied by Donald Nordlund (*C. marginiventris*) and Esmat Hegazi (*M. rufiventris*). We also thank Syngenta (Stein,Switzerland) for the weekly shipment of *Spodoptera littoralis* eggs and caterpillars. We are grateful to Ingrid Ricard and Anthony Davison for the log linear model construction and help with statistical analyses. This study was supported by the Swiss National Science Foundation (grants 31-46237-95 and 31-058865-99) and was a collaborative effort conducted in the context of the Swiss National Centre of Competence in Research "Plant Survival".

References

AGELOPOULOS, N. G. and KELLER, M. A. 1994a. Plant natural enemy association in the tritrophic system, *Cotesia rubecula-Pieris rapae*-Brassiceae (Cruciferae).1. Sources of infochemicals. *J. Chem. Ecol.* 20: 1725-1734.

AGELOPOULOS, N. G. and KELLER, M. A. 1994b. Plant natural enemy association in tritrophic system, *Cotesia rubecula-Pieris rapae*-Brassicaceae (Cruciferae).3. Collection and identification of plant and frass volatiles. *J. Chem. Ecol.* 20: 1955-1967.

AUGER, J., LECOMTE, C., PARIS, J., and THIBOUT, E. 1989. Identification of Leek-Moth and Diamondback-Moth frass volatiles that stimulate parasitoid, *Diadromus pulchellus*. *J. Chem. Ecol.* 15: 1391-1398.

BAEHRECKE, E. H., WILLIAMS, H. J., and VINSON, S. B. 1989. Electroantennogram responses of *Campoletis sonorensis* (Hymenoptera, Ichneumonidae) to chemicals in cotton (*Gossypium hirsutum*-L). *J. Chem. Ecol.* 15: 37-45.

BERNASCONI, M. L., TURLINGS, T. C. J., AMBROSETTI, L., BASSETTI, P., and DORN, S. 1998. Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entom. Exp. Appl.* 87: 133-142.

CORTESERO, A. M., DEMORAES, C. M., STAPEL, J. O., TUMLINSON, J. H., and LEWIS, W. J. 1997. Comparisons and contrasts in host-foraging strategies of two larval parasitoids with different degrees of host specificity. *J. Chem. Ecol.* 23: 1589-1606.

D’ALESSANDRO, M. and TURLINGS, T. C. J. 2005. *In Situ* modification of herbivore-induced plant odours: A novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. *Chem. Senses* 30: 739-753.

D’ALESSANDRO, M. and TURLINGS, T. C. J. 2006. Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *The Analyst* 131: 24–32.

DEGEN, T., DILLMANN, C., MARION-POLL, F., and TURLINGS, T. C. J. 2004. Genetic variability in herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiol.* 135: 1928-1938.

DE MORAES, C. M. and LEWIS, W. J. 1999. Analyses of two parasitoids with convergent foraging strategies. *J. Insect Behav.* 12: 571-583.

DICKE, M. 1999a. Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entom. Exp. Appl.* 91: 131-142.

DICKE, M. 1999b. Specificity of herbivore-induced plant defences, pp. 43-54, in D. J. Chadwick and J. A. Goode (eds.) Insect-Plant Interactions and Induced Plant Defence. Novartis Foundation, John Wiley and Sons, Chichester, UK.

DROST, Y. C., LEWIS, W. J., and TUMLINSON, J. H. 1988. Beneficial arthropod behavior mediated by airborne semiochemicals.5. Influence of rearing method, host plant, and adult experience on host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis*. *J. Chem. Ecol.* 14: 1607-1616.

DU, Y. J., POPPY, G. M., POWELL, W., PICKETT, J. A., WADHAMS, L. J., and Woodcock, C. M. 1998. Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *J. Chem. Ecol.* 24: 1355-1368.

ELLER, F. J., TUMLINSON, J. H., and LEWIS, W. J. 1992. Effect of host diet and preflight experience on the flight responses of *Microplitis croceipes* (Cresson). *Physiol. Entomol.* 17: 235-240.

ELZEN, G. W., WILLIAMS, H. J., and VINSON, S. B. 1984. Isolation and identification of cotton synomones mediating searching behavior by parasitoid *Campoletis sonorensis*. *J. Chem. Ecol.* 10: 1251-1264.

ELZEN, G. W., WILLIAMS, H. J., and VINSON, S. B. 1986. Wind-tunnel flight responses by Hymenopterous parasitoid *Campoletis sonorensis* to cotton cultivars and lines. *Entom. Exp. Appl.* 42: 285-289.

ELZEN, G. W., WILLIAMS, H. J., VINSON, S. B., and POWELL, J. E. 1987. Comparative flight behavior of parasitoids *Campoletis sonorensis* and *Microplitis croceipes*. *Entom. Exp. Appl.* 45: 175-180.

GEERVLIET, J. B. F., POSTHUMUS, M. A., VET, L. E. M., and DICKE, M. 1997. Comparative analysis of headspace volatiles from different caterpillar-infested or uninfested food plants of Pieris species. *J. Chem. Ecol.* 23: 2935-2954.

GEERVLIET, J. B. F., VET, L. E. M., and DICKE, M. 1996. Innate responses of the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae) to volatiles from different plant-herbivore complexes. *.J Insect Behav.* 9: 525-538.

GEERVLIET, J. B. F., VREUGDENHIL, A. I., DICKE, M., and VET, L. E. M. 1998. Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula*. *Entom. Exp. Appl.* 86: 241-252.

GOUINGUENE, S., DEGEN, T., and TURLINGS, T. C. J. 2001. Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecol.* 11: 9-16.

GOUINGUENE, S., PICKETT, J. A., WADHAMS, L. J., BIRKETT, M. A., and TURLINGS, T. C. J. 2005. Antennal electrophysiological responses of three parasitic wasps to caterpillar-induced volatiles from maize (*Zea mays*), cotton (*Gossypium herbaceum*), and cowpea (*Vigna unguiculata*). *J. Chem. Ecol.* 31: 1023-1038.

HEATH, R. R. and MANUKIAN, A. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plants using a charcoal-infused medium for air purification. *J. Chem. Ecol.* 18: 1209-1226.

HENDRY, L. B., GREANY, P. D., and GILL, R. J. 1973. Kairomone mediated host-finding behavior in parasitic wasp *Orgilus lepidus*. *Entom. Exp. Appl.* 16: 471-477.

HOBALLAH, M. E. F., TAMÒ, C., and TURLINGS, T. C. J. 2002. Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: Is quality or quantity important? *J. Chem. Ecol.* 28: 951-968.

HOBALLAH, M. E. F. and TURLINGS, T. C. J. 2005. The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. *J. Chem. Ecol.* 31: 2003-2018.

JONES, R. L., LEWIS, W. J., BOWMAN, M. C., BEROZA, M., and BIERL, B. A. 1971. Host-seeking stimulant for parasite of corn earworm - isolation, identification, and synthesis. *Science* 173, 842-843.

KRIPS, O. E., WILLEMS, P. E. L., GOLS, R., POSTHUMUS, M. A., GORT, G., and DICKE, M. 2001. Comparison of cultivars of ornamental crop *Gerbera jamesonii* on production of spider mite-induced volatiles, and their attractiveness to the predator *Phytoseiulus persimilis*. *J. Chem. Ecol.* 27: 1355-1372.

LEWIS, W. J. and JONES, R. L. 1971. Substance that stimulates host-seeking by *Microplitis croceipes* (Hymenoptera-Braconidae), a parasite of *Heliothis* species (Lepidoptera, Noctuidae). *Ann. Entomol. Soc. USA* 64: 471-473.

LEWIS, W. J. and TUMLINSON, J. H. 1988. Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* 331: 257-259.

LI, Y. S., DICKENS, J. C., and STEINER, W. W. M. 1992. Antennal olfactory responsiveness of *Microplitis croceipes* (Hymenoptera, Braconidae) to cotton plant volatiles. *J. Chem. Ecol.* 18: 1761-1773.

LOUGHRIN, J. H., MANUKIAN, A., HEATH, R. R., and TUMLINSON, J. H. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *J. Chem. Ecol.* 21: 1217-1227.

MCAUSLANE, H. J., VINSON, S. B., and WILLIAMS, H. J. 1991. Influence of adult experience on host microhabitat location by the generalist parasitoid, *Campoletis sonorensis* (Hymenoptera, Ichneumonidae). *J. Insect Behav.* 4: 101-113.

MCCALL, P. J., TURLINGS, T. C. J., LEWIS, W. J., and TUMLINSON, J. H. 1993. Role of plant volatiles in host location by the specialist parasitoid *Microplitis croceipes* Cresson (Braconidae, Hymenoptera). *J. Insect Behav.* 6: 625-639.

NORDLUND, D. A., LEWIS, W. J., and ALTIERI, M. A. 1988. Influences of plant-produced allelochemicals on the host/prey selction behavior of entomophagous insects, pp. 65-90, In P. Barbosa and D. K. Letourneau (eds.) Novel Aspects of Insect-Plant Interactions, John Wiley, New York, USA.

PARÉ, P. W. and TUMLINSON, J. H. 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiol.* 121: 325-331.

RAMACHANDRAN, R. and NORRIS, D. M. 1991. Volatiles mediating plant-herbivore-natural enemy interactions - Electroantennogram responses of Soybean Looper, *Pseudoplusia includens,* and a parasitoid, *Microplitis demolitor*, to green leaf volatiles. *J. Chem. Ecol.* 17: 1665-1690.

RAMACHANDRAN, R., NORRIS, D. M., PHILLIPS, J. K., and PHILLIPS, T. W. 1991. Volatiles mediating plant-herbivore-natural enemy interactions - Soybean Looper frass volatiles, 3-octanone and guaiacol, as kairomones for the parasitoid *Microplitis demolitor*. *J. Agricult. Food Chem.* 39: 2310-2317.

RAPUSAS, H. R., BOTTRELL, D. G., and COLL, M. 1996. Intraspecific variation in chemical attraction of rice to insect predators. *Biol. Control* 6: 394-400.

REDDY, G. V. P., HOLOPAINEN, J. K., and GUERRERO, A. 2002. Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *J. Chem. Ecol.* 28: 131-143.

SCHNEE, C., KÖLLNER, T. G., HELD, M., TURLINGS, T. C. J., GERSHENZON, J., and DEGENHARDT, J. A maize terpene synthase contributes to a volatile defense signal that attracts natural enemies of maize herbivores. *Proc. Natl. Acad. Sci. USA* (in press)

STEINBERG, S., DICKE, M., and VET, L. E. M. 1993. Relative importance of infochemicals from 1st and 2nd trophic level in long-range host location by the larval parasitoid *Cotesia glomerata*. *J. Chem.Ecol.* 19: 47-59.

TAKABAYASHI, J., DICKE, M., and POSTHUMUS, M. A. 1991. Variation in composition of predator-attracting allelochemicals emitted by herbivore-infested plants: relative influence of plant and herbivore. *Chemoecol.*2: 1-6.

TAMÒ, C. 2006. A comparative study on the plant odour preferences and learning ability of three solitary endoparasitoids of *Spodoptera* species: University of Neuchâtel.

TAMÒ, C., RICARD, I., HELD, M., DAVISON, A. C., and TURLINGS, T. C. J. A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours. *Animal Biol.* (in press).

TURLINGS, T. C. J. and BENREY, B. (1998). Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience* 5, 321-333.

TURLINGS, T. C. J., DAVISON, A. C., and TAMÒ, C. 2004. A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiol. Entomol.* 29: 45-55.

TURLINGS, T. C. J., LENGWILER, U. B., BERNASCONI, M. L., and WECHSLER, D. 1998. Timing of induced volatile emissions in maize seedlings. *Planta* 207: 146-152.

TURLINGS, T. C. J., SCHEEPMAKER, J. W. A., VET, L. E. M., TUMLINSON, J. H., and LEWIS, W. J. (1990a). How contact foraging experiences affect preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera, Braconidae). *J. Chem. Ecol.* 16: 1577-1589.

TURLINGS, T. C. J. and TUMLINSON, J. H. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proc. Nat. Acad. Sci. USA* 89: 8399-8402.

TURLINGS, T. C. J., TUMLINSON, J. H., ELLER, F. J., and LEWIS, W. J. 1991. Larval-damaged plants - source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. *Entom. Exp. Appl.* 58: 75-82.

TURLINGS, T. C. J., TUMLINSON, J. H., and LEWIS, W. J. 1990b. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.

TURLINGS, T. C. J., TUMLINSON, J. H., LEWIS, W. J., and VET, L. E. M. 1989. Beneficial arthropod behavior mediated by airborne semiochemicals.8. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *J. Insect Behav.* 2: 217-225.

TURLINGS, T. C. J., WAECKERS, F., VET, L. E. M., LEWIS, W. J., and TUMLINSON, J. H. 1993. Learning of host-finding cues by hymenopterous parasitoids, pp. 51-78, in D. R. Papaj and A. Lewis (eds.) Insect Learning: Ecological and Evolutionary Perspectives,. Chapman & Hall, New York, USA.

TURLINGS, T. C. J. and WÄCKERS, F. L. 2004. Recruitment of predators and parasitoids by herbivore-damaged plants, pp. 21-75, in R. T. Cardé and J. Millar (eds.) Advances in Insect Chemical Ecology, Cambridge University Press, UK.

VAUGHN, T. T., ANTOLIN, M. F., and BJOSTAD, L. B. 1996. Behavioral and physiological responses of *Diaeretiella rapae* to semiochemicals. *Entom. Exp. Appl.* 78: 187-196.

VET, L. E. M. and DICKE, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Ann. Rev. Entomol.* 37: 141-172.

VET, L. E. M. and GROENEWOLD, A. W. 1990. Semiochemicals and learning in parasitoids. *J. Chem. Ecol.* 16: 3119-3135.

VET, L. E. M., LEWIS, W. J., and CARDÉ, R. T. 1995. Parasitoid foraging and learning, pp. 65-101, in R. T. Cardé and W. J. Bell (eds.), Chemical Ecology of Insects 2, Chapman & Hall, New York, USA.

VINSON, S. B. 1984. Parasite-host relationships, pp. 205-233, in W. J. Bell and R. T. Cardé (eds.), Chemical Ecology of Insects, Chapman and Hall, London, UK.

WHITMAN, D. W. 1988a. Allelochemicals interactions among plants, herbivores, and their predators, pp. 11-64, in P. Barbosa and D. K. Letourneau (eds.), Novel Aspects of Insect-Plant Interactions, John Wiley, New York, USA.

WHITMAN, D. W. 1988b. Plant natural products as parasitoid cuing agents, pp. 386-396, in H. G. Cuttler (ed.) Biologically Active Natural Products Potential use in Agriculture, American Chemical Society, Washington, D. C.

WHITMAN, D. W. and ELLER, F. J. 1992. Orientation of *Microplitis croceipes* (Hymenoptera, Braconidae) to green leaf volatiles - Dose-response curves. *J. Chem. Ecol.* 18: 1743-1753.

Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time saving foraging strategy

Based on: Cristina Tamò, Lise-Lore Roelfstra, Guillame Suzanne and Ted C. J. Turlings*. Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time-saving foraging strategy, *Journal of Animal Ecology* (2006) 75, 1091-1099.

Journal of Animal Ecology 2006
75, 1091–1099

Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time-saving foraging strategy

CRISTINA TAMÒ, LISE-LORE ROELFSTRA, SUZANNE GUILLAUME and TED C. J. TURLINGS*

Université de Neuchâtel, Institut de Zoologie, Laboratoire d'entomologie évolutive, Case Postale 158, 2009, Neuchâtel, Switzerland

Summary

1. In studies on optimal foraging strategies, long-range decisions in the pursuit of resource are rarely considered. This is also the case for sympatric parasitoids, which may be confronted with the decision to accept or reject host larvae that are already parasitized by a competing species. They can be expected to reject already parasitized hosts if it is likely that they will lose the resulting intrinsic competition. However, examples of such interspecific host discrimination are rare.

2. We propose that parasitoids that are not egg-limited should reject inferior hosts only if it saves them time, and that this will be achieved mainly when the parasitoids are able to detect competitors from a distance. We tested this hypothesis using the sympatric parasitoids *Cotesia marginiventris* (Cresson) and *Campoletis sonorensis* (Cameron).

3. *C. sonorensis* was found to be the superior intrinsic competitor but, upon contact with a host larva, both wasps readily accepted hosts that had already been parasitized by the other species. However, in an olfactometer experiment, *C. marginiventris* females were found to strongly avoid the odour of their superior competitor.

4. These results are in accordance with a time optimization scenario, whereby the inferior competitor accepts competition if it costs only an egg, but avoids competition if it may save time that can be allocated to the search for more profitable hosts.

5. Models on host discrimination strategies in parasitoids had not yet considered discrimination from a distance. Long-range foraging decisions can also be expected for other organisms that have to choose between resources of varying suitability and profitability.

Key-words: competition, host discrimination, olfaction, optimal foraging, parasitoids.

Journal of Animal Ecology (2006) **75**, 1091–1099
doi: 10.1111/j.1365-2656.2006.01128.x

Introduction

Optimal foraging theory concerns the decisions that animals make while foraging for resources that contribute to their reproductive success. Parasitic wasps searching for suitable hosts for their offspring have been ideal models for such studies (Godfray 1994; Quicke 1997). Parasitoids frequently have to decide whether to lay eggs in already parasitized hosts, which may be inferior resources compared to unparasitized hosts. In case of multiparasitism by individuals of more than one solitary endoparasitoid species, usually only one individual will successfully develop inside the host, after having eliminated the other(s). Parasitoid larvae can combat each other through physical attack, physiological suppression or both (Salt 1961; Vinson 1972; Vinson & Iwantsch 1980b; Bai & Mackauer 1991; Tillman & Powell 1992a, 1992b; van Baaren & Nenon 1996). The species that lays its egg first can be expected to win the competition (Bautista & Harris 1997; Wallner, Weseloh & Grinberg 1982; Collier, Kelly & Hunter 2002), but often the survival among same-aged parasitoid larvae is found to be independent of the oviposition sequence (Mackauer *et al.* 1992). The outcome of competition may also depend on the time elapsed between the two parasitization events (Isenhour 1988; Baur & Yeargan 1995; De Moraes *et al.* 1999; Ueno 1999).

Correspondence: Ted C. J. Turlings, Université de Neuchâtel, Institut de Zoologie, Laboratoire d'entomologie évolutive, Case Postale 158, 2009 Neuchâtel, Switzerland.
E-mail: ted.turlings@unine.ch

If female wasps can distinguish between already parasitized and unparasitized larvae they may reject the parasitized hosts to avoid competition. Rejection of hosts already parasitized by conspecifics (intraspecific host discrimination) is common in larval parasitoids (van Alphen & Visser 1990). Kin selection is likely to be one of the driving forces that has led to the recognition and avoidance of hosts that are already parasitized by conspecifics. Indeed, the likelihood of avoidance of superparasitism increases with the relatedness of the potentially competing parasitoids (Marris, Hubbard & Scrimgeour 1996). Kinship does not play a role in the avoidance of multiparasitism and therefore, in the absence of relatedness, it is expected that competitive ability (chance of surviving the competition), eggload and time are the key factors that determine the incidence of host discrimination (van Lenteren 1981; van Alphen & Visser 1990). It has been a topic of considerable discussion whether eggload or time is the principal limiting factor of fitness in parasitoids (Rosenheim 1996; Sevenster, Ellers & Driessen 1998), but it is clear that in nature the average life span of solitary parasitoids is often too short to deposit all their eggs (Rosenheim 1999). Therefore selection should strongly favour time optimization in parasitoid foraging strategies.

Interspecific host discrimination (avoidance of multiparasitism) has been rarely observed and mainly for closely related species (van Lenteren 1981; Vet *et al.* 1984; van Baaren, Boivin & Nenon 1994; Royer *et al.* 1999; Agboka *et al.* 2002; Ardeh, de Jong & van Lenteren 2005), in which cases it could be an artefact of still using the same cues to avoid superparasitism. Van Alphen & Vissen (1990) argue convincingly that interspecific host discrimination should only evolve in inferior competitors. As some parasitoid species are indeed very poor competitors inside the host (intrinsic), why then is interspecific host discrimination rarely observed among sympatric, non-related parasitoids? Here we argue that most studies have not considered host discrimination from a distance.

Solitary endoparasitoids that use lepidopteran larvae as host usually carry ample eggs and time is therefore expected to be their most important limiting factor. Based on a simulation model, Turlings, van Batenburg & van Strien van Liempt (1985) suggest that as long as a female parasitoid is not egg-limited and oviposition is not overly time-consuming she should accept a host already parasitized by another species, even if the chance of offspring survival is small. Based on results of a field study on parasitoids of *Drosophila* larvae, Janssen (1989) drew a similar conclusion. Things would be different if host rejection would result in considerable time gain, time that could be used to find more suitable hosts (Turlings *et al.* 1985). Such a time gain could be achieved if already parasitized hosts are detected and rejected from a distance.

Long-distance avoidance of possible competitors was first reported by Price (1970), who found that in the three ichneumonid genera *Pleolophus*, *Endasys* and *Mastrus*, females avoid areas where other parasitoids had searched previously for hosts. Similarly, Janssen *et al.* (1995a, b) showed the ability of the parasitoid *Leptopilina heterotoma* to recognize and avoid patches with the superior competitor, *L. clavipes*, using volatiles substances. This avoidance occurs only when both hosts and competing parasitoids are present on the same patch and the competitors have had contact with the hosts. The exact source and identity of the avoidance-invoking odour remains unknown. The odours could be produced by parasitoids searching for hosts, by host larvae that are being attacked or they could be a side effect of the oviposition behaviour of the parasitoid (Janssen *et al.* 1995b). Interestingly, and in accordance with our hypothesis, *L. heterotoma* do not avoid laying eggs in hosts already parasitized by *L. clavipes*. Thus, Janssen and coworkers provide the first support for a time saving strategy. Such sophistication in parasitoid foraging behaviour can be expected only in closely coevolved systems, where parasitoids occupy similar niches.

Here we present a study on another closely coevolved system for which we specifically tested the hypothesis of time-saving competition avoidance. The solitary endoparasitoids *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) are the main parasitoid species that attack larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), one of the most important insect pests of different crops in the Americas (Sparks 1979; Andrews 1988; Ashley *et al.* 1989). *C. marginiventris* and *C. sonorensis* co-occur throughout North America and have been collected frequently in the same maize fields (Pair *et al.* 1986; Andrews 1988; Riggin *et al.* 1993; Molina-Ochoa *et al.* 2001; Hoballah *et al.* 2004) and are expected to have a long history together, which may have led to traits that reduce competition between the wasps. They both attack second and third instar larvae (Isenhour 1985; Jalali, Singh & Ballal 1987), but neither *C. marginiventris* nor *C. sonorensis* seems to avoid to lay eggs in larvae already parasitized by conspecifics or by other species (Rajapakse, Ashley & Waddill 1991; Rajapakse, Waddill & Ashley 1992; Baur & Yeargan 1995; Escribano *et al.* 2000).

Several studies have evaluated how *C. marginiventris* (Isenhour 1988; Rajapakse *et al.* 1991; Rajapakse *et al.* 1992; Escribano *et al.* 2000; Sallam, Overholt & Kairu 2002) and *C. sonorensis* (Vinson 1972; Vinson & Ables 1980a; Escribano *et al.* 2000) compete with other parasitoid species, but no information exists on the competition between the two. Depending on the species they were competing with, both species were found to be either superior or inferior intrinsic competitors (Miller 1977; Isenhour 1988; Rajapakse *et al.* 1991; Baur & Yeangan 1995; Escribano *et al.* 2000). All the larval instars of both *C. marginiventris* and *C. sonorensis* possess mandibles that potentially could be used to attack other parasitoid larvae (Boling & Pitre 1970; Wilson & Ridgway 1975). The developmental time of *C. marginiventris* and

C. sonorensis is similar and depends strongly on temperature (Wilson & Ridgway 1975; Kunnalaca & Mueller 1979; Isenhour 1986).

Like many other parasitoids of herbivores (Turlings & Benrey 1998; Dicke & Vet 1999; Turlings & Wäckers 2004b), *C. marginiventris* and *C. sonorensis* are both attracted to the odours produced by the plants attacked by their hosts (Elzen, Williams & Vinson 1983, 1984; Turlings, Tumlinson & Lewis 1990; McAuslane, Vinson & Williams 1991; Turlings, Davison & Tamo 2004a). However, nothing is known about the long-range interaction and possible interference in the hosts searching process between the two species.

Here we show that *C. sonorensis* has the best chance to survive in case of multiparasitism and that this is affected neither by the sequence in which the two species parasitize the same host nor by the time difference between the respective ovipositions. The competitive superiority of *C. sonorensis* was confirmed in cage studies with host larvae on small maize plants, in which the wasps could forage freely together. A six-arm olfactometer was used to investigate if the wasps respond to the odour of competing females, with the expectation that the inferior competitor might avoid the odour of other species to optimize its time allocation in favour of the search for the most suitable hosts. *C. marginiventris* was indeed found to be repelled by the odour of *C. sonorensis*.

Methods

INSECTS

The rearing colony of *C. marginiventris* originated from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi, USA). The *C. sonorensis* colony was established in 2001 from individuals collected from parasitized *S. frugiperda* larvae in maize fields in Mexico (Poza Rica, Veracruz, Mexico). That year the *C. marginiventris* was replenished with wasps from the same fields. Both parasitoids were reared on *S. littoralis*. For the rearing, 25 *S. littoralis* caterpillars (2–4 days old) were offered to two mated females (4–7 days old) for 3 h in a plastic container (9·5 cm diameter, 5 cm high). The parasitized caterpillars were then placed into transparent plastic boxes (15 × 13 × 5·5 cm) on a wheatgerm-based artificial diet until cocoon formation. Cocoons were kept in open Petri dishes, which were placed in Bugdorm-1 cages (30 × 30 × 30 cm) (MegaView Science Education Services Co. Ltd, Taiwan) at a sex ratio of 1 : 2 (male : female), with wet cotton wool and honey as a food source. Parasitized host larvae and adults of *C. marginiventris* were held until the experimental day in an incubator (25 °C and 16 L : 8D), whereas parasitized larvae and adults of *C. sonorensis* were held in the laboratory under ambient light and temperature conditions (19–24 °C). All experiments were conducted with 2–4-day-old naive mated females.

S. littoralis larvae were reared from eggs provided by Syngenta (Stein, Switzerland). The eggs were kept in the above-described incubator and after emergence larvae were placed on artificial diet at room temperature, until using them either for rearing or for the experiments.

PLANTS

For the cage experiment 10–11-day-old maize plants with four to five fully developed leaves (var. Delprim) were used, whereas 8–9-day-old maize plants of the same variety with three to four developed leaves were used for the olfactometer experiment. One seed (for the cage experiment) or two seeds (for the olfactometer experiment) were planted per pot (200 mL) in commercial soil (COOP, Switzerland) and grown in a climate chamber (23 °C, 60% r.h., 16D: 8 L, 50 000 L m^{-1} m^2). The day before an olfactometer experiment started, the plant pairs were transplanted into glass pots that fitted the olfactometer (250 mL, 4·5 cm diameter, 11 cm high).

INTRINSIC COMPETITION

To determine the intrinsic competitive ability of each species we set up the following experiment. Twenty-five second instar *S. littoralis* larvae were offered to two parasitoid females (2–4 days old) of one of the two parasitoid species in a plastic box (9·5 cm diameter, 5 cm high). If a female did not sting any larvae within 5 min, another female was used. As soon as stinging with the ovipositor was observed, the presumably parasitized larva was removed and transferred to another identical plastic box. After 25 larvae were parasitized they were either (1) kept on artificial diet or (2) offered to two parasitoid females of the other species after 5–10 min (0 days), 1 day or 2 days. During the second oviposition cycle the females were also observed and the multiparasitized larvae were removed immediately and transferred into a new plastic box with artificial diet. All the larvae that were assumed to have been singly parasitized or multiparasitized were then reared on artificial diet until cocoon development, in a separate box for each of the four treatments. The experiment was replicated six times for each combination of parasitoids and time delay between ovipositions. Unparasitized larvae were easily recognizable after 3–5 days, as they grew much larger than the parasitized larvae. They were counted and removed. The number of dead larvae was recorded daily, as were the number of cocoons of each parasitoids species that were formed.

CAGE EXPERIMENTS

To investigate possible competitive interactions in a simultaneous foraging situation, a second experiment was carried out where the females could interfere with each other either directly (disturbance, aggressive behaviour) or indirectly (interspecific host discrimination, multiparasitism, etc.). For each replication we used six cages Bugdorm-2 (60 × 60 × 60 cm) (MegaView Science Education Services Co. Ltd, Taiwan), each holding five pots containing a 10–11-day-old maize plant. Fifteen second instar *S. littoralis* larvae were placed on each plant (75 larvae per cage) and were allowed to feed during one night before releasing the wasps. Each cage received a different combination of parasitoids: either eight *C. marginiventris*, eight *C. sonorensis*, four *C. marginiventris* + four *C. sonorensis*, four *C. marginiventris*, four *C. sonorensis* or two *C. marginiventris* + two *C. sonorensis*. The parasitoids were left with the plants and the larvae for 24 h. The hosts were collected, counted and kept in plastic boxes with artificial diet (one per cage). The experiment was replicated three times. We checked daily for dead and unparasitized larvae, and for the emergence of the cocoons.

For both the above experiments, a *G*-test with William's correction was used to test for differences between the species in the number of cocoons, unparasitized larvae, dead larvae and larvae that were not found back (the latter only for the cage experiment). In cases of significance, a pairwise comparison between treatments was performed after the *G*-values were subjected to a Yates' continuity correction.

OLFACTOMETER ASSAYS

A six-arm olfactometer (Turlings *et al.* 2004a) was used to test if the female wasps responded to the odour females of the competing species or to the odour of conspecifics. The olfactometer consists of six odour vessels that are connected to the six arms of a central chamber in which the parasitoids can choose between different odours. In this apparatus both species have shown to be attracted readily to the odours of plants damaged by their hosts (personal observations). The experiments tested if this attraction of the parasitoids to the induced plant odours was affected by the presence of females of their own species or of the competing species. Three odour sources were prepared, each with two maize plants on which 20 second instar *S. littoralis* larvae were allowed to feed during one night; vessels holding these plants were alternated with empty (control) vessels. One h before an assay, 10 females of *C. marginiventris* were placed in an arm connected to a vessel with caterpillar-damaged plants; to another such arm 10 *C. sonorensis* females were added, and no wasps were added to the third arm. During the first 0·5 h the females were trapped in the arms using plugs of cotton to prevent them from either walking up into the trapping bulb or out of the arm into the choice chamber (see Turlings *et al.* 2004a for a detailed drawing of the olfactometer). In this manner, any odour emitted by the females could adsorb onto the glass in the arm. After 0·5 h the cotton plugs were removed and the normal experimental airflows were passed through all arms. The wasps remained in the arm and were given 0·5 h to settle in the trapping bulb. On each test day, three groups of six naive females of each species were released alternately in the choice chamber and their choices were recorded. Each group of females was given 0·5 h to make a choice and then removed. This experiment was replicated on 6 different days, with the different odours being introduced through different arms and alternating between the species that was first released in the olfactometer. A log-linear model assuming quasinormal distribution, thus allowing for overdispersion of the wasps, was used in statistical tests for odour preferences (Turlings *et al.* 2004a).

COLLECTION AND ANALYSES OF VOLATILES

During the 3-h bioassays, part of the volatile emissions from each odour source was collected by sucking 50% of the air flow out of each odour source vessel through a trapping filter containing 25 mg of 80–100 mesh Super Q adsorbent (Altech, Deerfield, IL, USA), as described by Turlings *et al.* (2004a). Immediately after each experiment, the volatiles collected on the filters were extracted with 150 µL of methylene chloride and two internal standards (n-octane and nonyl acetate, each 200 ng in 10 µL methylene chloride) were added to these extracts. Aliquots of these samples were analysed by gas chromatography and mass spectrometry, as described by Turlings *et al.* (2004a). Total quantities of the collected volatiles were calculated based on their peak areas compared to those of the internal standards. Analysis of variance (ANOVA) and Student–Newman–Keuls *post-hoc* tests were used to compare the total quantity of volatiles emitted among plant species.

Results

INTRINSIC COMPETITION

In all cases where the larvae were multiparasitized, significantly more *C. sonorensis* than *C. marginiventris* cocoons emerged from the parasitized larvae (Fig. 1). Larvae that were parasitized only by *C. sonorensis* yielded similar numbers of *C. sonorensis* cocoons as the multiparasitized larvae ($G_{adj.} = 3{\cdot}42$, $P = 0{\cdot}75$). In contrast, when larvae were parasitized by *C. marginiventris* only, significantly more *C. marginiventris* cocoons were produced than when larvae were multiparasitized ($G_{adj.} = 134{\cdot}28$, $P < 0{\cdot}001$). When the larvae were subjected to an oviposition by only one of the two species, significantly more *C. marginiventris* emerged than *C. sonorensis* ($G_{adj.} = 10{\cdot}11$, $P < 0{\cdot}01$). The number of parasitized larvae that died before forming a cocoon was also significantly different among treatments: there were fewer dead larvae when they were parasitized only once and in the cases of multiparasitism larval mortality increased with the number of days between the two parasitization events ($G_{adj.} = 16{\cdot}69$, $P < 0{\cdot}05$). The numbers of larvae that were found to be unparasitized (or survive parasitism) was significantly higher when they only had been stung by *C. sonorensis* ($G_{adj.} = 70{\cdot}20$, $P < 0{\cdot}001$).

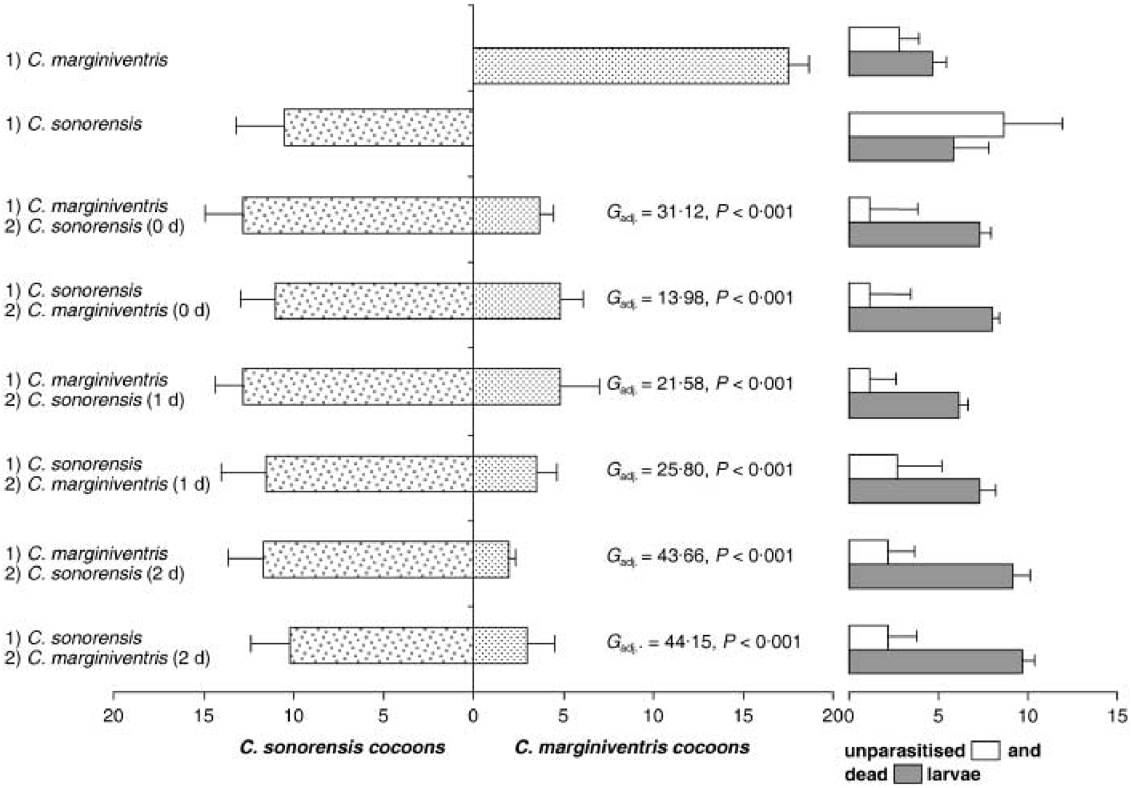


Fig. 1. Outcome of intrinsic competition experiments. Host larvae were parasitized by *C. marginiventris* and/or *C. sonorensis* and in case of parasitization by both species, the order in which the two species were allowed to oviposit and the time in between ovipositions varied (0, 1 or 2 days in between ovipositions). The bars on the left indicate the number of parasitoids that emerged from the host larvae (number of cocoons formed). Significant differences between the cocoons recorded for the two species are shown next to the bars. The bars on the right represent the larvae that died or survived parasitism.

CAGE EXPERIMENTS

In the cages where the two parasitoids foraged together significantly more *C. sonorensis* than *C. marginiventris* cocoons emerged from the larvae (Fig. 2: $G_{adj.} = 41\cdot85$, $P < 0\cdot001$); this was true in the cages with two *C. marginiventris* and two *C. sonorensis* females ($G_{adj.} = 21\cdot44$, $P < 0\cdot001$), as well as for the cages with four females of each species ($G_{adj.} = 19\cdot29$, $P < 0\cdot001$). The number of cocoons of *C. sonorensis* was significantly higher for the cage where four *C. sonorensis* females foraged alone compared with all the other treatments with *C. sonorensis* ($G_{adj.} = 13\cdot95$, $P < 0\cdot01$). Significantly fewer *C. marginivetris* cocoons were found in the cages where the two species were foraging together compared with the cages where *C. marginiventris* foraged alone ($G_{adj.} = 77\cdot13$, $P < 0\cdot001$), but no significant difference was found in the number of cocoons between cages with either four or eight females of this species foraging alone ($G_{adj.} = 0\cdot74$, $P = 0\cdot4$). There was no difference between *C. marginiventris* and *C. sonorensis* in parasitism effectiveness when they foraged alone ($G_{adj.} = 0\cdot01$, $P = 0\cdot9$).

The number of parasitized larvae that died before a cocoon was formed was significant different among treatments. Fewer larvae died from cages with only *C. sonorensis*, and larval mortality was highest for the cages in which four *C. marginiventris* and four *C. sonorensis* had foraged together ($G_{adj.} = 18\cdot70$, $P < 0\cdot01$). The number of unparasitized larvae was highest in the two treatments with only *C. marginiventris* ($G_{adj.} = 31\cdot82$, $P < 0\cdot001$). Finally, the number of larvae that were not found back was significantly higher for the cages with eight *C. sonorensis* ($G_{adj.} = 16\cdot99$, $P < 0\cdot01$).

OLFACTOMETER ASSAYS

Females of both species were readily attracted to the arms that carried the odour of host-infested plants (Fig. 3). *C. marginiventris* females rarely entered the empty arms or the arm with *C. sonorensis* females plus maize odour, whereas they often chose the arm containing only maize odour or the arm with maize odour combined with *C. marginiventris* females (Fig. 3a). The *C. sonorensis* females also rarely entered the empty arms, but they chose equally well among the arms containing maize odour, independently of the presence of other female wasps (Fig. 3b). No significant differences were found between the total amounts of volatiles produced by the induced maize plants present in the different arm (Fig. 3c; $P = 0\cdot22$).

Discussion

The intrinsic competition between *C. sonorensis* and *C. marginiventris* was almost always won by *C. sonorensis*, even if *C. marginiventris* had parasitized 2 days earlier

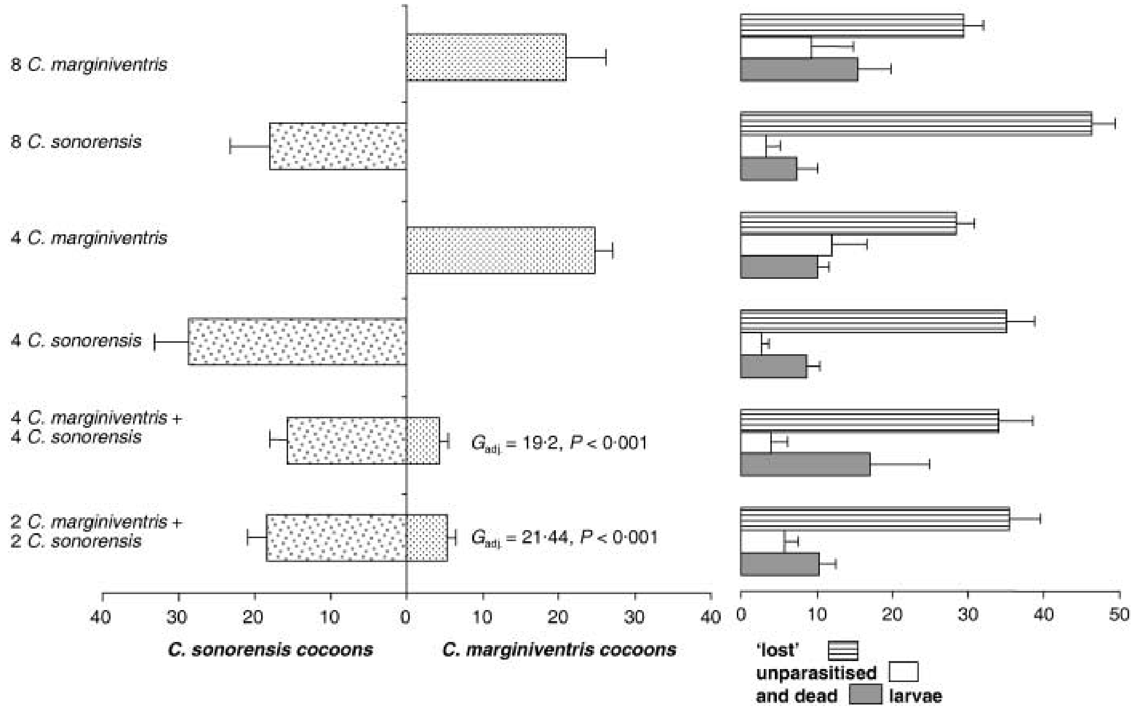


Fig. 2. Outcome of cage competition experiments. *C. marginiventris* and/or *C. sonorensis* wasps were placed for 24 h in cages with ample host larvae on maize plants. Numbers of wasps in the different cages varied as indicated. The bars on the left represent the number of parasitoids that emerged from the recollected host larvae (number of cocoons formed). Significant differences between the cocoons recorded for the two species are shown next to the bars. The bars on the right represent the larvae that were not found back, that died or that survived parasitism.

(Fig. 1). We did not establish whether this was a result of physical or physiological attack. However, cases of physical attack have been documented for *C. sonorensis* by Vinson & Ables (1980a). A closely related species, *C. perdistinctus*, employs both physical and physiological attack to suppress the larvae of *Cardiochiles nigriceps* in case of multiparasitism, depending on the time elapsed among the two parasitization events and on their sequence (Vinson 1972).

A large proportion of larvae was not successfully parasitized when *C. sonorensis* was the sole attacker (Fig. 1). This either means that there was not always an egg deposited when a sting was observed, or that some eggs or larvae did not survive inside the singly parasitized hosts. Therefore the competitive ability of *C. sonorensis* may have been under-estimated, because a certain proportion of the larvae that were assumed to be double-parasitized might not have contained a viable *C. sonorensis* larva. Indeed, *Spodoptera* species are not the most suitable hosts for *C. sonorensis*, as they may be able to resist parasitism by encapsulating a proportion of the parasitoid's eggs (Prevost, Davies & Vinson 1990; Cui, Soldevila & Webb 2000). In this context it should be noted that there was a consistent tendency that in the trials where *C. marginiventris* parasitized first, more *C. sonorensis* were produced. This suggests that *C. sonorensis* larvae may have benefited from the ability of *C. marginiventris* to suppress a host resistance factor. Various hymenopteran parasitoids, including *C. marginiventris* (Hamm, Styer & Lewis 1990; Styer, Hamm & Nordlund 1987), suppress host resistance by injecting a polydnavirus in a host together with an egg and a study by Cusson *et al.* (2002) suggest that the injection of a polydnavirus by one parasitoid species may help a second parasitizing species. Hence, *C. sonorensis* may benefit from the presence of *C. marginiventris* and there would be no reason for the former to avoid the latter. In fact, *C. sonorensis* can be expected to prefer hosts larvae that have already been parasitized by *C. marginiventris*, which could explain why the olfactometer arm with *C. marginiventris* attracted slightly more *C. sonorensis* (Fig. 3b).

The cage experiments showed that when *C. marginiventris* and *C. sonorensis* were alone their parasitism rates were comparable. However, when the two species of parasitoids searched together for the hosts in the same cage, the proportion of *C. marginiventris* emerging was drastically reduced (Fig. 2). This is due probably to the superiority of *C. sonorensis* in the intrinsic competition, but could also in part be the result from a reduced oviposition rate caused by direct interference between the adults. The latter possibility is indicated by the results from the olfactometer experiment. Both species were equally attracted to the induced odours produced by the maize plants under caterpillar attack, but the *C. marginiventris* females avoided the arms containing females of *C. sonorensis*. This implies that *C. marginiventris* uses volatile cues to avoid patches where *C. sonorensis* females are already searching for

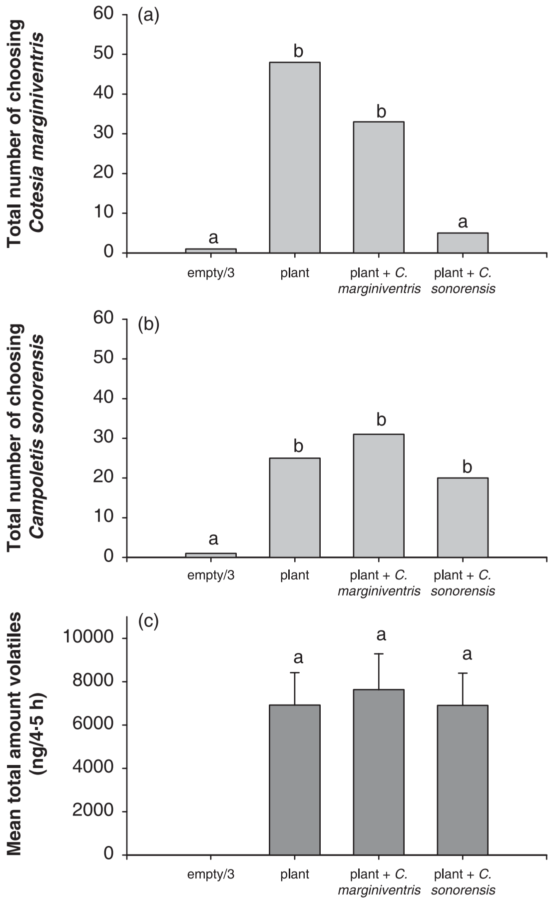


Fig. 3. Results from six-arm olfactometer experiments. Responses of females of (a) *C. marginiventris* or (b) *C. sonorensis* that were offered a choice between the odours of host-infested maize plants alone, host-infested maize plants plus 10 *C. marginiventris* females, host-infested maize plants plus 10 *C. sonorensis* females, and three olfactometer arms that carried clean air. The bars represent the totals of choices made over all replicates. The letters above the bars indicate significant differences calculated from a log-linear model, which used the values of each replicate ($n = 18$ groups of six wasps, $P < 0{\cdot}001$). Graph (c) shows the total amounts of volatiles collected for each odour source during the bio-assays ($n = 6$). No significant differences were found among the arms that carried the odour of an infested maize plant.

hosts. This is similar to the interaction between the two *Drosophila* parasitoids *Leptopilina heterotoma* and *L. clavipes* (Janssen *et al.* 1995a). The inferior competitor, *L. heterotoma*, avoids stinkhorn patches were *L. clavipes* are present. Janssen *et al.* (1995b) also found that *L. heterotoma* avoids stinkhorn patches with conspecifics. *C. sonorensis* and *C. marginiventris* did not avoid the odour of conspecifics.

As in the studies by Janssen and colleagues the long-range avoidance of heterospecific competitors must be due to a repellent odour. In our case it can be excluded that the repellence was due to a change in the odours produced by the herbivores in the presence of the competing parasitoid, as has been suggested in the avoidance of the *Drosophila* parasitoid *L. clavipes* by its competitor *L. heterotoma* (Janssen *et al.* 1995b). In our olfactometer assays, the plants and the hosts were separated from the parasitoids and there was no interaction with hosts possible.

Price (1970) noted that ichneumonid parasitoid females avoided areas that had previously been searched by conspecific and heterospecific parasitoids and suggested that this was due to the 'pungent' smell they emit. Ours appears to be the first study to demonstrate that indeed parasitoid-produced odours directly affect other members of the third tropic level. *C. sonorensis* is an ichneumonid and, as was the case for the wasps studied by Price (1970), produces a scent that is detectable by the human nose. Hence, an odourous signal is available that may be used by *C. marginiventris* to avoid its competitor. This avoidance of competition occurs only at a distance and not when a female contacts larvae that have already been parasitized. This may be because there are no contact signals that allow them to make the distinction between parasitized and unparasitized host, but given that various parasitoids can make such a distinction (van Alphen & Visser 1990), it is more likely that the wasps 'choose' to accept inferior hosts when they contact them. This is in accordance with our expectation that the wasps' foraging success is limited by time rather than by egg load. The wasps carry ample eggs and can waste some to poor-quality hosts. Moreover, an oviposition takes a few seconds at most, whereas the preceding location of the host can take considerably more time (personal field observations). Avoiding already parasitized hosts from a distance may therefore save them sufficient time to increase the likelihood of finding a larger number of more suitable hosts during their life span. Theoretical models used to predict whether or not parasitoids should discriminate between parasitized and unparasitized host have not yet considered discriminations from a distance (van Alphen & Visser 1990). These models predict that discrimination is more likely to occur if rejection time is much shorter than handling time (host recognition and oviposition time). Adding host recognition from a distance and subsequent host location time to the handling time in these models would greatly alter their outcome. Hence, interspecific host discrimination may be far more common than thus far assumed. Optimal foraging models developed for other systems should also consider the possibility of time saving foraging decisions from a distance.

Acknowledgements

The authors thank the members of the group led by Martine Rahier at the Institute of Zoology (University of Neuchâtel) for fruitful discussions and/or useful comments on the manuscript. Anthony Davison, Ingrid Ricard, and Matthias Held helped with the statistics. This work was supported by the Swiss National Science Foundation (grants 31-46237-95 and 31-058865-99) and was a collaborative effort conducted in the context of the Swiss National Centre of Competence in Research 'Plant Survival'.

References

Agboka, K., Schulthess, F., Chabi-Olaye, A., Labo, I., Gounou, S. & Smith, H. (2002) Self-, intra-, and interspecific host discrimination in *Telenomus busseolae* Gahan and T-isis Polaszek (Hymenoptera: Scelionidae), sympatric egg parasitoids of the African cereal stem borer *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae). *Journal of Insect Behavior*, **15**, 1–12.

van Alphen, J.J.M. & Visser, M.E. (1990) Superparasitism as an adaptive strategy for insect parasitoids. *Annual Review of Entomology*, **35**, 59–79.

Andrews, K.L. (1988) Latin-American research on *Spodoptera frugiperda* (Lepidoptera, Noctuidae). *Florida Entomologist*, **71**, 630–653.

Ardeh, M.J., de Jong, P.W. & van Lenteren, J.C. (2005) Intra- and interspecific host discrimination in arrhenotokous and thelytokous *Eretmocerus* spp. *Biological Control*, **33**, 74–80.

Ashley, T.R., Wiseman, B.R., Davis, F.M. & Andrews, K.L. (1989) The fall armyworm – a bibliography. *Florida Entomologist*, **72**, 152–202.

van Baaren, J., Boivin, G. & Nenon, J.P. (1994) Intraspecific and interspecific host discrimination in two closely related egg parasitoids. *Oecologia*, **100**, 325–330.

van Baaren, J. & Nenon, J.P. (1996) Intraspecific larval competition in two solitary parasitoids, *Apoanagyrus* (*Epidinocarsis*) *lopezi* and *Leptomastix dactylopii*. *Entomologia Experimentalis et Applicata*, **81**, 325–333.

Bai, B. & Mackauer, M. (1991) Recognition of heterospecific parasitism – competition between Aphidiid (*Aphidius ervi*) and Aphelinid (*Aphelinus asychis*) parasitoids of aphids (Hymenoptera, Aphidiidae, Aphelinidae). *Journal of Insect Behavior*, **4**, 333–345.

Baur, M.E. & Yeargan, K.V. (1995) Host selection and larval interactions among three primary parasitoids of *Plathypena scabra* (Lep. Noctuidae). *Entomophaga*, **40**, 357–366.

Bautista, R.C. & Harris, E.J. (1997) Effects of multiparasitism on the parasitization behavior and progeny development of oriental fruit fly parasitoids (Hymenoptera: Braconidae). *Journal of Economic Entomology*, **90**, 757–764.

Boling, J.C. & Pitre, H.N. (1970) Life history of *Apalantes marginiventris* with descriptions of immature stages. *Journal of the Kansas Entomological Society*, **43**, 465–470.

Collier, T., Kelly, S. & Hunter, M. (2002) Egg size, intrinsic competition, and lethal interference in the parasitoids *Encarsia pergandiella* and *Encarsia formosa*. *Biological Control*, **23**, 254–261.

Cui, L.W., Soldevila, A.I. & Webb, B.A. (2000) Relationships between polydnavirus gene expression and host range of the parasitoid wasp *Campoletis sonorensis*. *Journal of Insect Physiology*, **46**, 1397–1407.

Cusson, M., Laforge, M., Regniere, J., Beliveau, C., Trudel, D., Thireau, J.C., Bellemare, G., Keirstead, N. & Stolz, D. (2002) Multiparasitism of *Choristoneura fumiferana* by the ichneumonid *Tranosema rostrale* and the tachinid *Actia interrupta*: occurrence in the field and outcome of competition under laboratory conditions. *Entomologia Experimentalis et Applicata*, **102**, 125–133.

De Moraes, C.M., Cortesero, A.M., Stapel, J.O. & Lewis, W.J. (1999) Intrinsic and extrinsic competitive interactions between two larval parasitoids of *Heliothis virescens*. *Ecological Entomology*, **24**, 402–410.

Dicke, M. & Vet, L.E.M. (1999) Plant–carnivore interactions: evolutionary and ecological consequences for plant, herbivore and carnivore. *Herbivores: Between Plants and Predators* (eds H. Olf, B.K. Brown & R.H. Drent), pp. 483–520. Blackwell, Oxford.

Elzen, G.W., Williams, H.J. & Vinson, S.B. (1983) Response by the parasitoid *Campoletis sonorensis* (Hymenoptera, Ichneumonidae) to chemicals (synomones) in plants – implications for host habitat location. *Environmental Entomology*, **12**, 1872–1887.

Elzen, G.W., Williams, H.J. & Vinson, S.B. (1984) Isolation and identification of cotton synomones mediating searching behavior by parasitoid *Campoletis sonorensis*. *Journal of Chemical Ecology*, **10**, 1251–1264.

Escribano, A., Williams, T., Goulson, D., Cave, R.D. & Caballero, P. (2000) Parasitoid–pathogen–pest interactions of *Chelonus insularis*, *Campoletis sonorensis*, and a nucleopolyhedrovirus in *Spodoptera frugiperda* larvae. *Biological Control*, **19**, 265–273.

Godfray, H.C.J. (1994) *Parasitoids: Behavioural and Evolutionary Ecology*. University Press, Princeton, NJ.

Hamm, J.J., Styer, E.L. & Lewis, W.J. (1990) Comparative virogenesis of filamentous virus and polydnavirus in the female reproductive-tract of *Cotesia marginiventris* (Hymenoptera, Braconidae). *Journal of Invertebrate Pathology*, **55**, 357–374.

Hoballah, M.E., Degen, T., Bergvinson, D., Savidan, A., Tamò, C. & Turlings, T.C.J. (2004) Occurrence and direct control potential of parasitoids and predators of the fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico. *Agricultural and Forest Entomology*, **6**, 83–88.

Isenhour, D.J. (1985) *Campoletis sonorensis* (Hym. Ichneumonidae) as a parasitoid of *Spodoptera frugiperda* (Lep. Noctuidae): host stage preference and functional response. *Entomophaga*, **30**, 31–36.

Isenhour, D.J. (1986) Developmental time, adult reproductive capability, and longevity of *Campoletis sonorensis* (Hymenoptera, Ichneumonidae) as a parasitoid of fall armyworm, *Spodoptera frugiperda* (Lepidoptera, Noctuidae). *Annals of the Entomological Society of America*, **79**, 893–897.

Isenhour, D.J. (1988) Interactions between 2 hymenopterous parasitoids of the fall armyworm (Lepidoptera, Noctuidae). *Environmental Entomology*, **17**, 616–620.

Jalali, S.K., Singh, S.P. & Ballal, C.R. (1987) Studies on host age preference and biology of exotic parasite, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *Entomon*, **12** (1), 59–62.

Janssen, A. (1989) Optimal host selection by *Drosophila* parasitoids in the field. *Functional Ecology*, **3**, 469–479.

Janssen, A., van Alphen, J.J.M., Sabelis, M.W. & Bakker, K. (1995a) Odor-mediated avoidance of competition in *Drosophila* parasitoids – the ghost of competition. *Oikos*, **73**, 356–366.

Janssen, A., van Alphen, J.J.M., Sabelis, M.W. & Bakker, K. (1995b) Specificity of odor mediated avoidance of competition in *Drosophila* parasitoids. *Behavioral Ecology and Sociobiology*, **36**, 229–235.

Kunnalaca, S. & Mueller, A.J. (1979) Laboratory study of *Apanteles marginiventris* (Hymenoptera, Braconidae), a parasite of Green Cloverworm (Lepidoptera, Noctuidae). *Environmental Entomology*, **8**, 365–368.

van Lenteren, J.C. (1981) Host discrimination by parasitoids. *Semiochemicals, Their Role in Pest Control* (ed. D.A. Nordlund), pp. 153–179. Wiley, New York.

Mackauer, M., Bai, B., Chow, A. & Danyk, T. (1992) Asymmetric larval competition between 2 species of solitary parasitoid wasps – the influence of superparasitism. *Ecological Entomology*, **17**, 233–236.

Marris, G.C., Hubbard, S.F. & Scrimgeour, C. (1996) The perception of genetic similarity by the solitary parthenogenetic parasitoid *Venturia canescens*, and its effects on the occurrence of superparasitism. *Entomologia Experimentalis et Applicata*, **78**, 167–174.

McAuslane, H.J., Vinson, S.B. & Williams, H.J. (1991) Stimuli influencing host microhabitat location in the parasitoid *Campoletis sonorensis*. *Entomologia Experimentalis et Applicata*, **58**, 267–277.

Miller, J.C. (1977) Ecological relationships among parasites of *Spodoptera praefica* (Lepidoptera-Noctuidae). *Environmental Entomology*, **6**, 581–585.

Molina-Ochoa, J., Hamm, J.J., Lezama-Gutierrez, R., Lopez-Edwards, M., Gonzalez-Ramirez, M. & Pescador-Rubio, A. (2001) A survey of fall armyworm (Lepidoptera: Noctuidae) parasitoids in the Mexican states of Michoacan, Colima, Jalisco, and Tamaulipas. *Florida Entomologist*, **84**, 31–36.

Pair, S.D., Raulston, J.R., Sparks, A.N. & Martin, P.B. (1986) Fall Armyworm (Lepidoptera, Noctuidae) parasitoids – differential spring distribution and incidence on corn and sorghum in the Southern United States and Northeastern Mexico. *Environmental Entomology*, **15**, 342–348.

Prevost, G., Davies, D.H. & Vinson, S.B. (1990) Evasion of encapsulation by parasitoid correlated with the extent of host hemocyte pathology. *Entomologia Experimentalis et Applicata*, **55**, 1–10.

Price, P.W. (1970) Trail odors – recognition by insects parasitic on cocoons. *Science*, **170**, 546–547.

Quicke, D.J. (1997) *Parasitic Wasps*. Chapman & Hall, London.

Rajapakse, R.H.S., Ashley, T.R. & Waddill, V.H. (1991) Interspecific competition between parasitoids of the fall armyworm, *Spodoptera frugiperda* (Smith, Je) (Lepidoptera, Noctuidae). *Insect Science and its Application*, **12**, 473–480.

Rajapakse, R.H.S., Waddill, V.H. & Ashley, T.R. (1992) Effect of host age, parasitoid age and temperature on interspecific competition between *Chelonus insularis* Cresson, *Cotesia marginiventris* Cresson and *Microplitis manilae* Ashmead. *Insect Science and its Application*, **13**, 87–94.

Riggin, T.M., Espelie, K.E., Wiseman, B.R. & Isenhour, D.J. (1993) Distribution of fall armyworm (Lepidoptera, Noctuidae) parasitoids on 5 corn genotypes in South-Georgia. *Florida Entomologist*, **76**, 292–302.

Rosenheim, J.A. (1996) An evolutionary argument for egg limitation. *Evolution*, **50**, 2089–2094.

Rosenheim, J.A. (1999) The relative contributions of time and eggs to the cost of reproduction. *Evolution*, **53**, 376–385.

Royer, L., Fournet, S., Brunel, E. & Boivin, G. (1999) Intra- and interspecific host discrimination by host-seeking larvae of coleopteran parasitoids. *Oecologia*, **118**, 59–68.

Sallam, M.N., Overholt, W.A. & Kairu, E. (2002) Intraspecific and interspecific competition between *Cotesia flavipes* and *Cotesia sesamiae* (Hymenoptera: Braconidae), gregarious larval endoparasitoids of lepidopteran stemborers. *Biocontrol Science and Technology*, **12**, 493–506.

Salt, G. (1961) Competition among insect parasitoids. *Symposia of the Society for Experimental Biology*, XV. *Mechanism in Biology Competition* (ed. F.L. Milthorpe), pp. 96–119. Academic Press, London.

Sevenster, J.G., Ellers, J. & Driessen, G. (1998) An evolutionary argument for time limitation. *Evolution*, **52**, 1241–1244.

Sparks, A.N. (1979) Review of the biology of the fall armyworm (Lepidoptera, Noctuidae). *Florida Entomologist*, **62**, 82–87.

Styer, E.L., Hamm, J.J. & Nordlund, D.A. (1987) A new virus associated with the parasitoid *Cotesia marginiventris* (Hymenoptera, Braconidae) – replication in noctuid host larvae. *Journal of Invertebrate Pathology*, **50**, 302–309.

Tillman, P.G. & Powell, J.E. (1992a) Interspecific discrimination and larval competition among *Microplitis croceipes*, *Microplitis demolitor*, *Cotesia kazak* (Hym, Braconidae), and *Hyposoter didymator* (Hym, Ichneumonidae), parasitoids of *Heliothis virescens* (Lep, Noctuidae). *Entomophaga*, **37**, 439–451.

Tillman, P.G. & Powell, J.E. (1992b) Intraspecific host discrimination and larval competition in *Microplitis croceipes*, *Microplitis demolitor*, *Cotesia kazak* (Hym, Braconidae) and *Hyposoter didymator* (Hym, Ichneumonidae), parasitoids of *Heliothis virescens* (Lep, Noctuidae). *Entomophaga*, **37**, 229–237.

Turlings, T.C.J. & Benrey, B. (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience*, **5**, 321–333.

Turlings, T.C.J., Davison, A.C. & Tamo, C. (2004a) A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiological Entomology*, **29**, 45–55.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, **250**, 1251–1253.

Turlings, T.C.J., van Batenburg, F.D.H. & van Strien van Liempt, W. (1985) Why is there no interspecific host discrimination in the 2 coexisting larval parasitoids of *Drosophila* species – *Leptopilina heterotoma* (Thomson) and *Asobara tabida* (Nees). *Oecologia*, **67**, 352–359.

Turlings, T.C.J. & Wäckers, F.L. (2004b) Recruitment of predators and parasitoids by herbivore-damaged plants. *Advances in Insect Chemical Ecology* (eds R.T. Cardé & J. Millar), pp. 21–75. Cambridge University Press, New York.

Ueno, T. (1999) Multiparasitism and host feeding by solitary parasitoid wasps (Hymenoptera: Ichneumonidae) based on the pay-off from parasitized hosts. *Annals of the Entomological Society of America*, **92**, 601–608.

Vet, L.E.M., Meyer, M., Bakker, K. & Vanalphen, J.J.M. (1984) Intraspecific and interspecific host discrimination in *Asobara* (Hymenoptera) larval endo-parasitoids of Drosophilidae – comparison between closely related and less closely related species. *Animal Behaviour*, **32**, 871–874.

Vinson, S.B. (1972) Competition and host discrimination between 2 species of tobacco budworm parasitoids. *Annals of the Entomological Society of America*, **65**, 229–246.

Vinson, S.B. & Ables, J.R. (1980a) Interspecific competition among endo-parasitoids of tobacco budworm larvae Lep. Noctuidae. *Entomophaga*, **25**, 357–362.

Vinson, S.B. & Iwantsch, G.F. (1980b) Host suitability for insect parasitoids. *Annual Review of Entomology*, **25**, 397–419.

Wallner, W.E., Weseloh, R.M. & Grinberg, P.S. (1982) Intrinsic competition between *Apanteles melanoscelus* Hym, Braconidae and *Rogas lymantriae* Hym. Braconidae reared on *Lymantria dispar* Lep, Lymantriidae. *Entomophaga*, **27**, 99–103.

Wilson, D.D. & Ridgway, R.L. (1975) Morphology, development, and behavior of immature stages of parasitoid, *Campoletis sonorensis* (Hymenoptera-Ichneumonidae). *Annals of the Entomological Society of America*, **68**, 191–196.

Received 19 October 2005; revised version accepted 9 May 2006

Conclusions

In chapter 1 it is shown that the six-arm olfactometer is a very effective tool to test the relative attractiveness of different odour sources to parasitic wasps. Female wasps responded extremely well in tests where they were offered a single odour source as well as in tests with multiple choices. The responses of wasps released in groups were the same as those released individually. The females did not attract or repel each other, but males were attracted into arms in which females had been released. Dose response tests with varying numbers of plants or host larvae on plants revealed that the wasps responded in a dose related manner, thus showing that the system is well suited to measure relative attractiveness. The clear choices of the insects among six possibilities provided substantial statistical power. Gas chromatographic analyses of sampled air revealed clean and effective odour trapping, which largely facilitates the comparison of results from behavioural assays with the actual blends of volatiles that were emitted by the various odour sources.

By studying the long-range attraction among conspecifics of the same or of different sex in chapter 2 we found that *C. marginiventris* males were strongly attracted to the odour of their conspecific females, whereas males were not attracted to other males, and females were not attracted to either sex. For *M. rufiventris* the attraction of males to females was weak but significant and again no attraction was found in all the other combinations tested. The attraction of *C. marginiventris* and *M. rufiventris* males to conspecific females suggests that females of these two species produce a volatile sex pheromone. Males of both species were found not to be attracted to host-induced plant odours and this lack of attraction was not changed by an experience with these induced odours in the presence of conspecific females. Hence, males of these two species do not make use of plant volatiles for mate finding and do not use learning as a means to associate potential mates with an experienced odour.

In chapter 3 it was found that the three wasp species responded differently to the induced odours of maize, cotton and cowpea. Naïve *C. marginiventris* and *C. sonorensis* choose equally among the odours of the three plants, whereas naïve *M. rufiventris* tended to prefer the odour of maize. After conditioning *C. marginiventris* always preferred the odour of the plant species that they had experienced, but the conditioned *M. rufiventris* showed an even stronger preference for maize odours, independently of the plant they experienced. *C. sonorensis* did not show a change in its preference after conditioning. These results seem to imply that different generalist parasitoids employ different foraging strategies and that associative learning is not necessarily part of it.

The first aim of chapter 4 was to test if preimaginal or emergence experiences affect the odour preferences of adult females of *C. marginiventris*. No such effect was found, as *C. marginiventris* females that were reared from hosts fed on different diets (artificial diet, maize, cotton or cowpea) did not show any differences in their preferences for induced odours of the three plants that were tested. The responses were independent of the plant that they were reared on. In this chapter we also found that naïve females and females with an oviposition experience on one of the three plants species would start to parasitize sooner and more larvae on one of the plant species than on artificial diet. No differences were found for the wasps experienced on a particular plant in how they parasitized on the different plant species, implying that, at short range, plant stimuli enhance oviposition drive, but that this is the result of priming (increasing “motivation”) rather than associative learning. However,females with an oviposition experience on artificial diet subsequently showed an increase in parasitism rate on all diets, suggesting that they did learn to associate the unnatural substances of the artificial diet with host presence.

In chapter 5 we studied the relative importance of host-derived and plant-derived volatiles for the attraction of *C. marginiventris* and *M. rufiventris*. Naïve *C. marginiventris* were found to be highly responsive to maize plants with fresh damage (which typically release green leaf volatiles). Wasps became more responsive to the odour of plants with older damage (which release specific herbivore-induced volatiles) after experiencing these odours during an encounter with hosts. In contrast, naïve *M. rufiventris* responded best to the complete blend of plant-produced volatiles, while experience with hosts on a damaged plant rendered this wasp more responsive to volatiles that are directly associated with the herbivores and/or their by-products. Volatiles coming from the faeces of its hosts seem to play an important role in guiding experienced *M. rufiventris* to plants infested by its host. Although both wasps have similar host ranges they clearly employ different strategies to find these hosts.

In chapter 6 we studied the competition between the sympatric larval parasitoids *Cotesia marginiventris* and *Campoletis sonorensis*. It was shown in no choice and in choice experiments that *C. sonorensis* always out competes *C. marginiventris* in multiparasitised hosts, independently of the sequence in which the two species parasitize a host and of the time difference between the respective ovipositions. In the six-arm olfactometer, *C. marginiventris* females avoided the odour of *C. sonorensis* females, which could be a foraging strategy that may help the wasp to reduce negative fitness effects of the intrinsic competition. This so-called counter-balanced competition may help the two species of wasps to co-exist and may increase their joint effectiveness as control agents.

Outlook

Overall, it can be concluded that the novel six-arm olfactometer can be used as a bio-assay tool that can rapidly provide more insight in the intricate use of volatile cues by parasitoids to find hosts and mates. The parasitoids studied here appear to use female-produced sex pheromones, but not plant odours, to locate mates. Further research should be conducted to determine if only virgin females release the pheromones, as is indicated by the results for *M. rufiventris*. Identification of such pheromones may be useful to monitor for parasitoid presence in the field and in studies on parasitoid population structures.

Generally, this work also highlights that different parasitoid species with similar host ranges and life cycles can show considerable differences in their responses to host-induced plant odours. These differences were most evident in how experiences with hosts and associated odours affected the responses of the females to induced plants odours. We should therefore be cautious in generalising the strategies that parasitoids have evolved to optimise host microhabitat location and it is important to recognize that each species needs to be studied separately to understand its specific behaviour. This is particularly important if we wish to optimise the effectiveness of parasitoids in biological pest control. In this context, the surprising results obtained for *M. rufiventris* are highly illustrative. Against expectations, this wasp appears to learn specific host-related cues during an encounter with hosts. Further work will have to reveal what the exact source of these cues is and, ideally, identify the volatile substances that are implicated.

An additional complicating factor was revealed in the study on the interactions between the sympatric and competing parasitoid species. Such interactions have to be taken into account for a full understanding of the foraging decisions made by parasitoids and if one wishes to maximise their effectiveness as biological control agents. Other biotic (e.g. pathogens) and abiotic (e.g. water stress on plants) factors can be expected to also have profound effects on the signals that the parasitoids have available to them and on how they exploit them. With this in mind, it seems pertinent that studies like the ones presented here are followed-up by field experiments in which the resulting hypotheses be tested. Without understanding how the numerous and complex interactions among the different actors of these systems are translated in the field we can only keep speculating on the ecological and evolutionary implications of our findings. Moreover, in order to exploit some of the findings from these studies for the biological control of pest insects, it is essential to identify the key volatiles that are responsible for the attraction of the parasitoids to host habitats. In the cases were these volatiles are produced by plants under attack by the pest, modern methods are now available to manipulate and enhance the production of the attractants. In that way, the mutualistic interaction that has evolved between the first and the third trophic level could be exploited for our own benefit.

Acknowledgements

My infinite gratitude to Mum and Dad for their endless love and support,
to Dario for being so patient, cheerful and supportive and to Emma for existing.

All present and past members of the LEAE/E-vol for sharing with me this very nice period of my life. Cri F. and Mary, it was wonderful to share the same office, thank you for being so sweet, Mary at the beginning of my PhD you learnt me everything, Cri F. you gave me plenty of advices on how to finally finish it!
Matthias, Nicolas, Thomas thank you for your generous help and all your statistical, technical and informatics advices.

Ted, thank you for accepting me as PhD Student, for supporting me and for “translating and re writing” my thesis in real English! You didn’t even killed me when I broke the first exemplar of the six arm olfactometer!
Betty also contributed to the final corrections of the thesis and gave me the opportunity to continue to speak Spanish at least a little bit, it was very nice and useful.

Martine gave support and provided some of the infrastructure needed for the experiments.

Yves thank you for taking care of all parasitoids and for the nice parasitoids picture you took.

Several other people took care of the parasitoids during my thesis and I would like to thank them all: Claire, Katja, Cristina M., Liselore, Audrey, Magali.

A. C. Davison and Ingrid Ricard developed statistical model for the data obtained with the six arm olfactometer. Jacqueline Moret provided very useful advices and practical help for other statistical analysis.

I would like to thank my thesis examiners: Louise Vet, Wolfgang Nentwig and Patrick Guerin.

Finally, thank you also to numerous people outside the university for providing helpful support in many ways, in particular, Michela Balmelli, Cristina Marazzi, Stéphanie Wyss and Aysim Yilmaz.

Curriculum vitae

Cristina Tamò Zürcher

Private address:
Pl. de la Cathédrale 8
1005 Lausanne
Switzerland
Tel.: +41 (0)21 311 00 76

Date and Place of Birth:	28 October 1971, Locarno (TI)
Nationality:	Swiss
Civil status:	married
Languages:	Italian, French, German, English, Spanish

Education

January 2000 - December 2005 PhD in Sciences, University of Neuchâtel, Switzerland, "A comparative study on the plant odour preferences and learning ability of three solitary endoparasitoids of *Spodoptera* species"

January 1999 – December 1999 Post graduate course in "Systematics and management of biodiversity",University of Lausanne, Switzerland, Thesis: "Parasitism of non-target lepidopteran species by *Trichogramma brassicae* (Bedzenko) (Hymenoptera: Trichogrammatidae) a biological control agent used against, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae)"

April 1996 – September 1996 MSc in Entomology, ETH of Zürich, Switzerland, "The effects of the induced odours of maize on the development of *Spodoptera littoralis (*Lepidoptera: Noctuidae*)*"

October 1990 – March 1996 Biology Degree with specialisation in Ecology and Ethology, ETH of Zürich, Switzerland

Professional experience

Since January 2003 “Scientific Coordinator” for Division III Biology and Medicine of the Swiss National Science Foundation, Berne, Switzerland.

January 2000 – December 2002 Teaching assistant, practical courses in “Chemical Ecology”, “Animal Ecology” and “Statistic for Biologist”

March 1998 – October 1998 Scientific collaborator at the Swiss Federal Research Station for vegetal production of Changins (RAC), Switzerland

July 1997 – September 1997 Research assistant and volunteer coordinator of a research study on two sea turtles species (*Eretmochelys imbricata* and *Chelonia Mydas*) and the bottlenose dolphin (*Tursiops truncates*) at Las Coloradas, Biosphere Reserve of Rio Lagartos, Yucatan, Mexico (Tethys Research Institute)

January 1997 – June 1997 Assistant at the Applied Entomology group at the ETH Zürich

Selected oral and poster presentations in Congresses

1. Tamò C., L. Roelfstra, G. Suzanne and T.C.J. Turlings (2003) Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid is in accordance with optimal foraging theory (poster presentation)
Biology 03, Swiss Society of Zoology and Botany, October 2003, Zürich, Switzerland

2. Tamò C., L. Roelfstra, G. Suzanne and T.C.J. Turlings (2003) Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid is in accordance with optimal foraging theory (oral presentation)
XIII International Entomophagous Insects Workshop, July 2003 Tucson, USA

3. Tamò, C., and T.C.J. Turlings (2002) A comparison of naïve and conditioned responses of two endoparasitoids of lepidopteran larvae to host-induced plant odours (oral presentation)
VIII European Workshop on Insect Parasitoids, Community Ecology, September 2002, Tours, France

4. Tamò, C., and T.C.J. Turlings (2002) A comparison of naïve and conditioned responses of three endoparasitoids of lepidopteran larvae to host-induced plant odours (oral presentation)
XIX Annual Meeting of the International Society of Chemical Ecology, August 2002, Hamburg, Germany

5. Tamò C. and T.C.J. Turlings (2002) A comparison of naïve and conditioned responses of three endoparasitoids of lepidopteran larvae to host-induced plant odours (oral presentation)
Biology 02, Swiss Society of Zoology and Botany, February 2002, Bern, Switzerland

6. Tamò C. and T.C.J. Turlings (2001) A comparison of naïve and conditioned responses of two endoparasitoids of lepidopteran larvae to host-induced plant odours (oral presentation)
III Cycle Romand Workshop and NCCR Plant Survival International Conference 2001 (Exploitation of chemical plant defenses by insects), September 2001, Neuchâtel, Switzerland

7. Tamò C. and T.C.J. Turlings (2001) A comparison of naïve and conditioned responses of three endoparasitoids of lepidopteran larvae to host-induced plant odours (poster presentation)
Biology 01, Swiss Society of Zoology and Botany, February 2001, Neuchâtel, Switzerland

8. Tamò C. and T.C.J. Turlings (2000) A six-arm olfactometer that allows for simultaneous observation of insect attractance and odour trapping (oral presentation)
III Cycle Romand Workshop and NCCR Plant Survival International Conference 2000 (Parasitoids: their biology, ecology and applications), September 2000, Neuchâtel, Switzerland

Publications

1. Tamò C., Ricard I., Held M. and Turlings T.C.J., 2006. A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours, Animal Biology, 56: 205-220.

2. Tamò C., Roelfstra L., Suzanne G. and. And Turlings T.C.J., 2006. Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time saving foraging strategy, Journal of Animal Ecology, 75: 1091-1099.

3. Turlings T.C.J., Davison A. C. and Tamò C., 2004. A six arm olfactometer permitting simultaneous observation of insect attraction and odour trapping, Physiological Entomology, 29 (1): 45-55.

4. Hoballah M. E., Degen T., Bergvinson D., Savidan A., Tamò C. and Turlings, T. C. J. 2004. Occurrence and direct control potential of parasitoids and predators of fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico, Agricultural and Forest Entomology, 6: 83-88.

5. Fritzsche Hoballah M.E., Tamò C. and Turlings T. C. J., 2002. Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid Cotesia marginiventris: Is quality or quantity important?, Journal of Chemical Ecology, 28(5): 951-968.

Continued education

Lectures followed within the PhD framework of the NCCR "Plant Survival in Natural and Agricultural Ecosystems" at the University of Neuchâtel:

1. "How to make scientific presentations and poster interesting? ", Prof. Dr. Jeremy Mc Neil, University of Laval, Canada, May 2002

2. "Effective Public Speaking", Prof. Marcel Lucien Goldschmit, EPFL, Switzerland, April-June 2002

3. "Statistique II: L'analyse des données en Ecologie", Prof. Dr. Diethart Matthies, University of Marburg, Germany, January 2002

4. "Statistique I: Introduction à l'Analyse des données pour Biologistes, Dr. Anne-Catherine Favre, University of Neuchâtel, January 2002